

MICROANATOMICAL STUDY ON THE EYES OF THE
LONE STAR TICK AND THE SCREWWORM FLY
WITH RELATED ELECTROPHYSIOLOGICAL STUDIES

By

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To my two precious daughters,
Emily and Colby,
without whom my life would have little meaning.

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PREFACE

The experimental data presented in this dissertation deal with two uniquely different photoreceptors, the tick eye and the insect eye. For the purposes of clarity, the current study has been subdivided into the following three chapters: Chapter I, The Microanatomy of the Eye of the "Lone Star Tick," Amblyomma americanum (L.); Chapter II, The Microanatomy of the Eye of the "Screwworm Fly," Cochliomyia hominovorax (Coquerel), and Chapter III, The Spectral Sensitivity of the Compound Eye of Cochliomyia hominovorax (Coquerel).

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The electron microscope was used to detail the microanatomy of the eyes of the "lone star tick," Amblyomma americanum (L.), and the "screwworm" fly, Cochliomyia hominivorax (Coquerel).

The eyes of the "lone star tick" consist of a cuticular lens and 30-40 underlying photoreceptor neurons. The lens contains bundles of specialized lenticular pore canals believed to function as light or wave guides. The photoreceptor neurons possess the microanatomical structures common to all rhabdomeric photoreceptors. The simplicity of the tick eye is believed to be a primitive condition and is the first arhabdomate eye described for the phylum Arthropoda. The photoreceptor neurons of Amblyomma show affinities with the arhabdomate eyes of the flatworms and snails.

The microanatomy of the peripheral retina and lamina of the "screwworm" fly, Cochliomyia hominivorax,

is similar to the other genera of cyclorrhaphan Diptera published. The eyes of irradiated and unirradiated flies were examined with electron microscopy. Irradiated flies showed a number of abnormalities not encountered in the unirradiated flies. Irradiated flies showed a large increase in retinular cell vacuolation, increased numbers of trachea in the peripheral retina, abnormally shaped rhabdomeres, decreased numbers of rhabdomeric microvilli, and abnormal central cell transitions. The function and origin of the Semper cells are discussed. The Semper cells are believed to have several functions. The most important function is to maintain the optically important trapezoidal pattern of the rhabdomeres and central cavity of the open rhabdom. The central cells (R7 and R8) have two types of rhabdomeres: The microvilli of the first type are orthogonal and the microvilli of the second are parallel. The pigment "granules" of previous authors are actually vacuoles filled with pigment crystals.

The electrophysiological method of equal response was used to determine the spectral sensitivity of the eye of irradiated screwworm flies. The eye was maximally sensitive at 490 nm with a secondary peak at 350 nm and a small "pseudopeak" at 625 nm. The visual sensitivity of irradiated mass reared screwworm flies exhibited considerable weekly variation, some weeks flies were as much as 10 times more sensitive than other weeks, when measured with the ERG at 530 nm.

CHAPTER I

THE MICROANATOMY OF THE EYE OF THE "LONE STAR TICK," Amblyomma americanum L.

Introduction

Ticks are extremely important pathophaerous agents. The lone star tick, Amblyomma americanum (L.), is an economically and medically important pest of wildlife, livestock and man. It ranges from central Texas throughout southcentral and southeastern United States north to Maryland and Pennsylvania (Cooley & Kohls, 1944; Bishopp & Trembley, 1945). A. americanum is an important vector of Rocky Mountain spotted fever (Rivers & Horsfall, 1959), Q fever, and is known to produce tick paralysis in man.

In general, tick physiology and biology have received considerable attention from Hoogstral (1970) in Africa and Sonenshine, Hair and Semtner in the United States. Studies by Sonenshine (Sonenshine et al., 1966; Sonenshine & Levy, 1971) and by Semtner et al. (Semtner et al., 1971a; Semtner et al., 1971b; Semtner & Hair, 1973a; Semtner & Hair, 1973b; Semtner et al., 1973) on the biology and ecology of Amblyomma americanum are particularly valuable. However, very few studies have included work on the photobiology of

ticks. This is difficult to understand in view of several studies indicating the importance of photoperiod in diapause, oviposition and questing behavior. McEnroe and McEnroe (1973) studied the questing behavior of Dermacentor variabilis (Say) and found that a photostimulus is necessary to initiate questing behavior. The ovipositional pattern of two ticks, Anocenter nitens Neumann and A. maculatum Koch, were shown to be highly sensitive to photoperiod (Wright, 1969; Wright, 1971). Photoperiod was also shown to be the critical factor in initiation of diapause in Dermacentor albipictus (Packard) (Wright, 1969) and Dermacentor variabilis (Smith & Cole, 1941). In addition most taxonomic keys to the hard ticks contain couplets that separate genera on the basis of the presence or absence of eyes.

No adequate research is available on the function and morphology of the eye of Ixodid ticks. Part of the problem with prior anatomical studies has been that they were done with light microscopy. Many of the characteristic structures of photoreceptor cells are beyond the resolving power of the light microscope and therefore fail to detail adequately the fine structure and make a determination possible as to whether or not a neuron could function as a photoreceptor.

This research was done with the electron microscope to investigate and detail the structure of the eye of Amblyomma americanum and to use the microanatomical analysis for determination of the eye as a functioning photoreceptor.

Literature Review

Two types of eyes are present in the arthropods: compound eyes consist of from several to several thousand repeating units known as ommatidia and small unicorneal eyes termed ocelli. The eyes of Amblyomma americanum are of the second or unicorneal type. Unicorneal eyes have received little attention in the literature due in part to the difficulty encountered in working with such small structures. The size and simplicity of these structures however make them of potential importance in neurophysiological vision studies and well suited to electron micrographic techniques.

With the exception of the compound eyes of Limulus chelicerate eyes are typically unilenticular ocelli. The anatomy and microstructure of these ocelli are very similar throughout the chelicerates. The lens is biconvex with an underlying vitreous body composed of a single layer of transparent cells. The photosensitive or retinular cells are organized into a cup-shaped retina. The closely packed microvilli characteristic to all arthropod retinular cells are always orientated perpendicular to the light path (Miller, 1960; Eakin, 1965). The microvilli of each cell form isolated units known as rhabdomeres or when combined with the microvilli from one or more other retinular cells they form a rhabdom.

Limulus has paired compound eyes as well as a pair of unicorneal ocelli and a rudimentary median eye. The ocelli consist of a biconvex lens and an underlying cup-shaped retina. Three types of retinular cells are present in the retina. The microvilli of the first type form single-layered rhabdomeres and the microvilli of the other two form double-layered rhabdoms. Rhabdoms consisting of two layers of microvilli are of two types: (1) "self-rhabdoms" in which both layers of microvilli arise from a single cell; (2) those rhabdoms in which the microvilli of two cells form a rhabdom (Nolte & Brown, 1971).

The phalangids are probably closely related to the acarines (van der Hammen, 1968). They have typical unilenticular ocelli consisting of a lens and an underlying retina. The photoreceptor cells of the retina are organized into units consisting of 4 retinula cells surrounding a central rhabdom. Each retinula cell contributes microvilli to the central rhabdom. The cytoplasm of the retinular cells contains many mitochondria, prominent Golgi, multivesicular bodies and endoplasmic reticula. The rhabdoms are highly ordered and repetitive with an organization very characteristic of the pattern found in compound eyes (Curtis, 1970).

Machan (1966) studied the structure of the lateral and median ocelli of three species of scorpions. The ocelli of scorpions consist of a biconvex corneal lens and a retinal layer of photoreceptor cells forming a cup-shaped

retina. This study was conducted with the light microscope and provides little information on the microstructure of the rhabdomeres or rhabdoms.

The two families of spiders that have been studied in detail with the electron microscope are the jumping spiders (Eakin & Brandenberger, 1971; Land, 1969) and the wolf spiders, Lycosa (Melamed & Trujillo-Cenoz, 1966; Baccetti & Bedini, 1964). The eyes of these two families of spiders are very similar and typical of those of the other chelicerates. The lenses are biconvex cuticular thickenings and possess highly ordered cup-shaped retinas. The rhabdomeres of both families are made up of microvilli and show a highly ordered repetitive pattern. Eakin and Brandenberger (1971) divided the sensory cells of the anterior median (AM) eyes into 4 regions: (1) a distal portion bearing the rhabdomeric microvilli, (2) an intermediate cytoplasmic segment, (3) a basal soma containing the nucleus, and (4) the long neurite that enters the optic nerve. The cell body or soma of the sensory cells of the other 6 eyes lies directly behind the vitreous body distad to the rhabdomeric microvilli.

The eyes of 2 acarine species have been anatomically studied. The eyes of these 2 species, Trombicula autumnalis Shaw and Tetranychus urticae Koch, show a similar anatomical organization.

T. autumnalis has 2 pair of eyes, the anterior pair with biconvex lenses and a posterior pair with simple convex lenses (Jones, 1950).

The eyes of I. urticae were studied by Mills (1973). The anterior pair have biconvex lenses consisting of a thin stratified external layer and a thick interior made up of 25-30 cuticular layers. The simple convex lens of the posterior pair of eyes is a thin "hemi-ellipsoidal shell" of cuticular material. Both pair of eyes share a common "eye-manifold" consisting of 15 retinular cells; five beneath the anterior eyes and 10 beneath the posterior eyes. The microvilli of the retinular cells lie within cup-shaped invaginations of the cell membrane termed "retinular-cups" by Mills. Three retinular cells of the anterior eyes and 8 retinular cells of the posterior eye have double rows of adjoining microvilli that form single fused rhabdoms similar to the "self-rhabdom" of Limulus. The other retinular cells have single rows of microvilli forming simple rhabdomeres.

Three anatomical studies of ticks have included work done on the eye. These prior studies, however, provide little information on the function of the eyes of ticks since they utilized light microscopy. The first person to detail the histology of the tick eye was Bonnet (1907). He described perpendicular striae accentuated by black pigment in the lens. Gossel (1935) studied the eyes of 6 species of Ixodid ticks and showed them to consist of a convex lens with underlying unipolar neurons. The eye of Dermacentor andersoni Stiles was treated briefly by Douglas (1943).

Eight species of eyeless ticks were studied by Binnington (1972). He found unipolar neurons in three of the ticks and believed them to be photoreceptor cells. He also found that removal of the lateral "eye" in Argas persicus Oken impeded phototaxis. All eight species studied had optic ganglia and optic nerves of similar morphology.

Horridge (1965) stated that the "aberrant eyes of ticks" do not fit into the category of arthropod photo-receptors.

Methods and Materials

Ticks utilized in this study were obtained from two sources. Larval and adult ticks were collected near Otter Creek, Florida, by dragging a 3-ft.-square "flag" over infested vegetation. Larval, nymphal, and adult ticks were also obtained from a colony maintained at the USDA-ARS, Insects Affecting Man and Animals Laboratory, Gainesville, Florida. These ticks were collected by personnel of the laboratory as adults and subsequently fed on a dog. The engorged ticks were held in a chamber maintained at high relative humidity. Each generation was reestablished with wild-caught adults (USDA Rearing Bulletin).

Ticks were fixed in gluteraldehyde-paraformaldehyde prepared according to Karnovsky (1965) and were then submerged in fixative and cut into three pieces. The opisthosoma was cut off behind the third pair of legs and discarded.

The podosoma was then cut into two pieces along the midline. This dissection facilitated the penetration of fixative and subsequent solutions.

Pieces of podosoma containing the eyes were placed in fresh fixative for 6-7 hours at room temperature to complete fixation. The pieces were washed in three changes of 0.1 M cacodylate buffer (pH 7.2) and post-fixed for 12 hours in 2% osmium tetroxide in 0.2 M cacodylate buffer at 4°C. Following post fixation the pieces were rinsed in 0.1 M cacodylate buffer prior to dehydration. Dehydration was accomplished at 5-minute intervals in a series of 25, 50, 75% ethanol at 4°C. The tissue was held in 2% uranyl acetate in 75% ethanol at 4°C for 3 hours to improve contrast. Two-10 minute changes of 100% ethanol and two subsequent 15 minute changes of acetone preceded infiltration with Spurr's plastic (Spurr, 1969). Tissue was held in 50% plastic in acetone for 1 hour and in 100% plastic for 24 hours at room temperature prior to polymerization in a 60° oven.

Silver to gold sections were cut using a duPont diamond knife on a Porter-Blum MT-2 ultramicrotome after thick sectioning (1 micron) brought the region of the eye to the block face. Thin sections were placed on 75-mesh copper grids covered with a Formvar film. The sections were post-stained with uranyl acetate for 10 minutes and lead citrate for 2-4 minutes (Reynolds, 1963) prior to examination with

either a Hitachi HU11C or HU11E electron microscope at 75 kV.

Results

The eyes of Ixodid ticks are located on the lateral margins of the scutum unlike other arthropods where the eyes are located on the head. In Amblyomma americanum the eyes consist of 30-40 unipolar photoreceptor neurons (Fig. 1). This pattern is the same for the larva, nymph, and adult tick. In each succeeding stage the eye becomes larger but the anatomy and microstructure remain the same. The eye of the larval tick contains approximately 25-30 neurons and is approximately one-fourth the size of the adult. Sections of a larval tick eye were used in Plates I and II to provide an overall view of the eye and the individual photoreceptor neurons. Plates I and II are electron micrographs of whole eyes. The orientation of the section may be determined by using the orientation lines provided on the plates themselves. One line (D-V) indicates the dorsal-ventral axis and the perpendicular line (L) indicates the midline-lateral aspect. The lenticular pore canals follow a curved path and converge in an area above the photoreceptor cells. Lines inscribed on the longitudinal axes of the pore canals would converge on a point in the microvillar region (region A) of the eye.

The cuticular lens is roughly biconvex and deviates only slightly from a simple convex configuration (Fig. 1, Plate I B). A slight internal bulge is present and is located on the ventral portion of the inner lens and the second convex curve of the lens. The internal bulge of the lens is always located proximal to the microvilli of the photoreceptor neurons (Plate I B). The pore canals of the lens are always perpendicular to the longitudinal axes of the photoreceptor neurons. The pore canals (PC) of the scutum are oriented in the dorsal-ventral axis (compare with the pore canals of the lens). The exocuticle of the lens (EXO) is darker in appearance than the endocuticle (ENDO) (Plate I B).

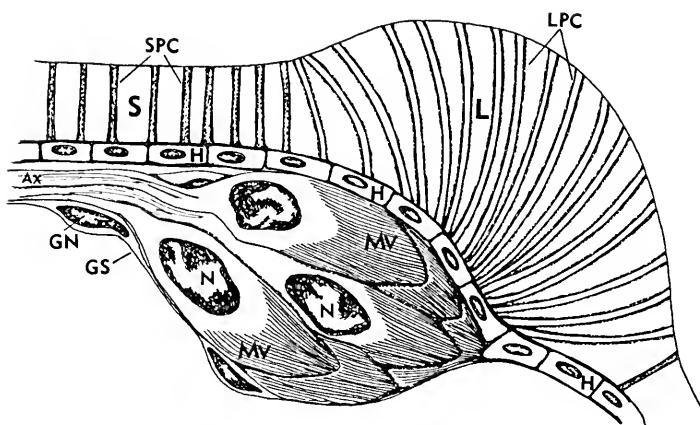
The photoreceptor neurons of the eye are connected to the optic lobes of the brain by the optic ganglion (Plate I A). Each photoreceptor neuron contributes a single axonal neurite to the optic nerve. The number of photoreceptor neurons per eye can therefore be determined by counting the number of axons in the optic nerve (Plate I A).

The individual photoreceptor neurons do not vary in structure with regard to sex, age, or stage. An isolated neuron is indistinguishable from any other neuron within a single eye. The photoreceptor neuron has been divided into 4 regions for descriptive purposes: (1) a distal segment, region A, characterized by the presence of numerous

Figure 1. Schematic diagram of an eye of Amblyomma americanum. Ax, axon; GN, glial nucleus, GS, glial sheath; H, hypodermis; L, lens; LPC, lenticular pore canals; Mv, microvilli of photoreceptor neuron; N, nucleus; S, scutum; SPC, scutellar pore canals.

Figure 2. Schematic diagram of 1 photoreceptor neuron of Amblyomma americanum. A, region characterized by numerous microvilli; B, region containing numerous mitochondria and intracellular channels; C, soma containing the nucleus; D, proximal axon; Ax, axon; CV, coated vesicles; GC, glial cell; GL, glycogen; GN, glial nucleus, Go, Golgi, M, mitochondria; Mv, microvilli.

1.



2.

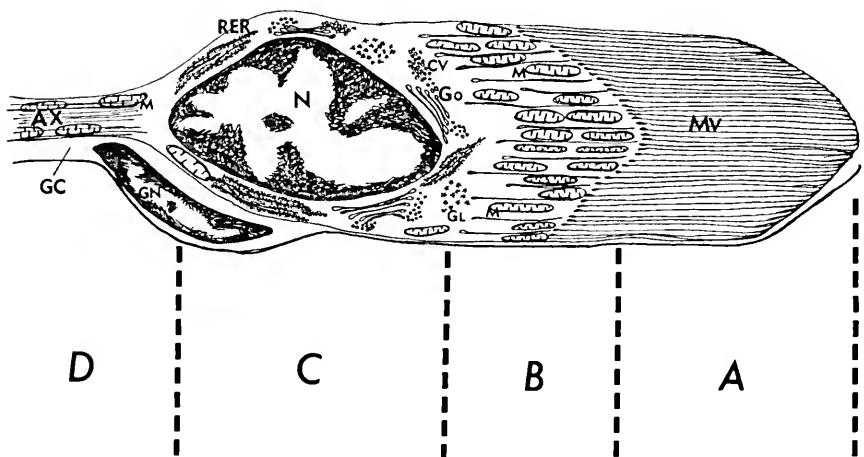


Plate I. Amblyomma americanum larva

- A. Oblique section of an optic nerve. Receptor cell axons (Ax) are invested by glial elements (arrows). The optic nerve lies directly beneath the scutum (Sc) and hypodermal cells (HC). X5890

- B. Oblique section of an eye. Beneath the lens (L) is the hypodermis (H) and 4 photoreceptor cells. Each photoreceptor cell has a prominent soma containing the nucleus (N) and numerous terminal microvilli (Mv). X3800 Note internal lenticular bridge (B), the deviation from simple convex configuration, and orientation of pore canals (PC).



microvilli, (2) an intermediate region containing numerous mitochondria and intracellular channels, region B, (3) a basal soma, region C, containing the nucleus, and (4) region D, a proximal axonal neurite that together with the other axonal fibers forms the optic nerve (Fig. 2). Three of these regions are shown in cross section in Plate V B. In addition to the photoreceptor neurons the eye is invested by a tunic of glial cells (Fig. 1, Plate V B). The membranous windings of the glial cells, the mesaxons (MA), glial cytoplasm (arrows), and an extracellular glial sheath (ES) isolate the neurons of the eye from the haemocoel. Often the cytoplasm of the glial cells contains electron dense opaque bodies (OB) and multivesicular bodies (MVB) (Plate V B).

Lens

The lenses of arthropod eyes contain very few structural features and in this respect the lens of Amblyomma americanum is unique. Unlike other lenses it has many pore canals in the transparent matrix of the scutellar cuticle. The pore canals (Plate III A) of the lens are organized into bundles of 30-60. These bundles of pore canals condense and their diameter becomes smaller as they approach the hypodermis (H). The number of pore canals per bundle also decreases as they near the hypodermis. This decrease is

Plate II. Amblyomma americanum larva

- A. Longitudinal section of an eye. Photoreceptor neurons have 4 distinct regions: a distal segment of microvilli, region A; an intermediate cytoplasmic segment containing many mitochondria, region B; a soma containing the nucleus (N), region C; and region D, a long axon (Ax). Note the orthogonal orientation of microvilli shown by arrows (CX and LX). X3800
- B. Longitudinal section of a portion of an eye (post-stained with barium permanganate). Microvilli of several cells are oriented orthogonally (arrows CX and LX). Four regions of the eye are shown (A, B, C, and D). X3800

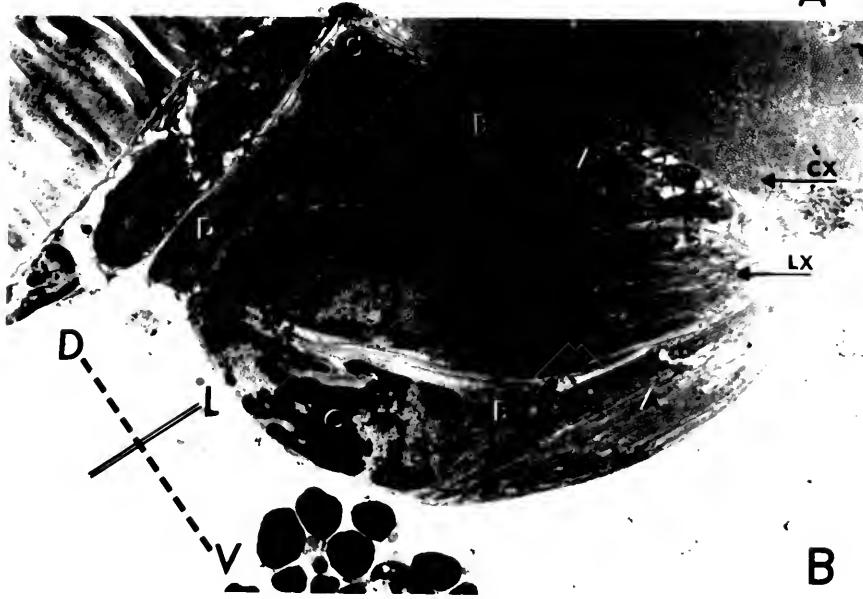


Plate III. Amblyomma americanum adult

A. Oblique cross section of the lens. The pore canals (PC) of the lens are organized into bundles. X3800 The arrows indicate fusion of pore canals.

B. Oblique section of lenticular pore canals (PC).
X3800

C. Longitudinal section of scutellar pore canals (PC). X3800

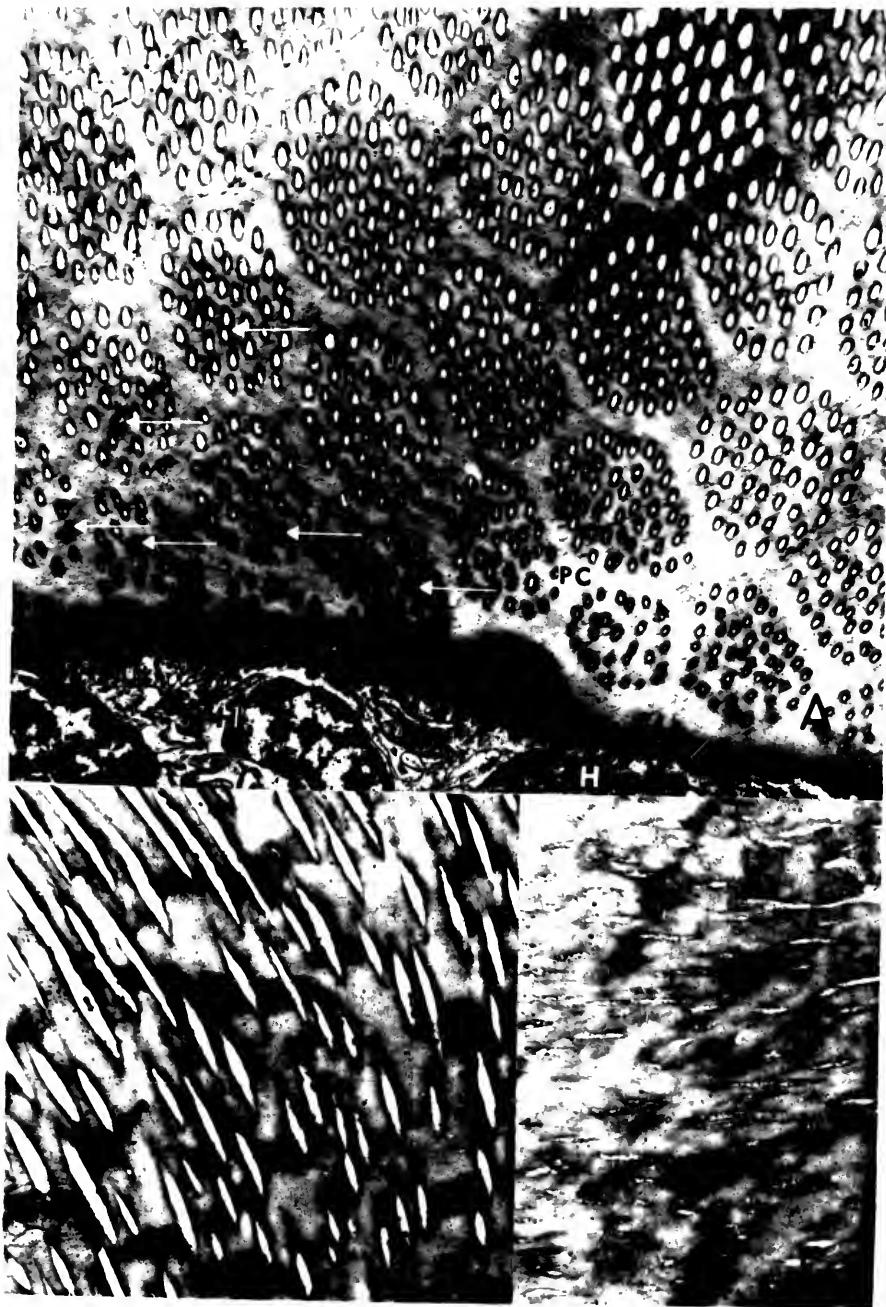
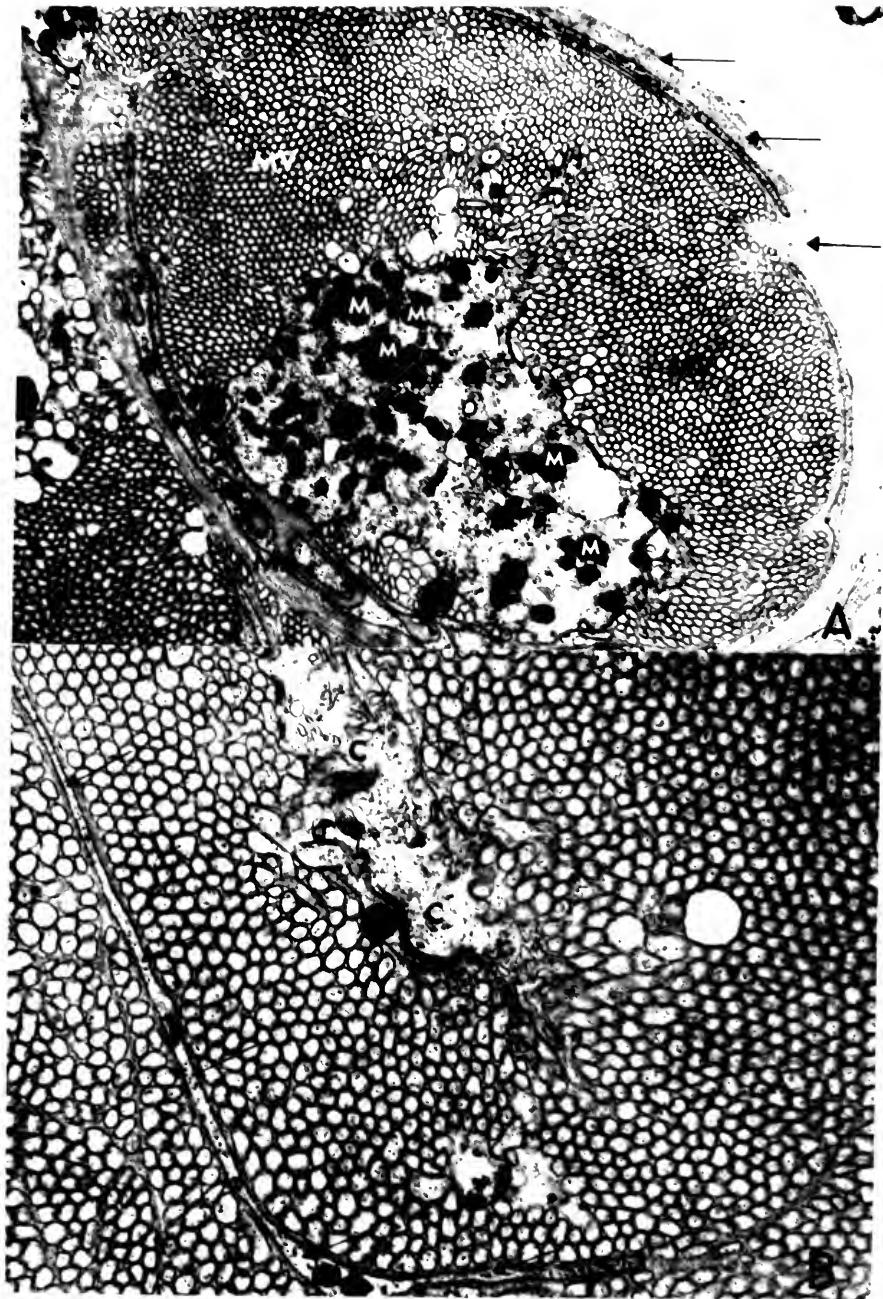


Plate IV. Amblyomma americanum adult

A. Cross section of terminal microvilli (Mv) bearing region of photoreceptor cell. Glial investiture of the photoreceptor cells consists of mesaxons (Ma) and an extracellular sheath (arrows). Neural cytoplasm contains numerous mitochondria (M) in this area. X11970

B. Cross section of terminal microvilli at higher magnification. Photoreceptor cytoplasm (C) and glial cell cytoplasm (arrows) shown. X22800



due to the regular fusion of pore canals (arrows). In Plate III A, pore canal bundles are demonstrated in cross section. This electron micrograph is oriented so the hypodermis underlying the lens is on the bottom and the top of the micrograph is laterad. The pore canal bundles diminish markedly in diameter as they near the hypodermis and several pore canals are sectioned at the point of fusion (arrows).

The lenticular pore canals and scutellar pore canals differ radically in size in the adult and the nymph. The lenticular pore canals are considerably larger (Plate III B) than the scutellar pore canals (Plate III C). Plate III B illustrates the curvilinear path taken by the pore canals of the lens.

Hypodermis

A cellular epidermis, the hypodermis (H), lies directly beneath the lens of the eye (Plate I B). The hypodermis is one cell layer thick and rests upon an amorphous basal basement membrane. The scutellar hypodermis (H) (Plate IX A) and the lenticular hypodermis (H) (Plate I B) are indistinguishable and no apparent lenticular hypodermal modifications were observed.

Retinular Cells

Region A. Microvilli

The distal portion of the photoreceptor neurons bears thousands of parallel microvilli (Fig. II, Plate II A & B). By counting the number of microvilli per square unit on a micrograph estimates of the number of microvilli per photoreceptor were calculated. These estimates ranged between 7,000 and 13,000 per photoreceptor cell. The microvilli are oriented perpendicular to the path of light as in all photoreceptors studied. The microvillar-bearing membrane of the neuron is dome-shaped (Fig. 2) and cross sections of this region often show a central portion of cytoplasm with microvillar cross sections encircling it (Plate IV A). The microvilli are independent and free within the glial investment of the neurons (Plate IV A). The microvilli are tightly packed within the mesaxonal investment (Plate VI B, V A) but are not bonded to one another by tight junctions as in other chelicerate eyes. The microvilli are typically blind-ended evaginations of the distal membrane of the neuron. Plate V A shows the tips of the photoreceptor microvilli.

Region B. Intermediate region of cytoplasm

The neural zone directly proximal to the terminal microvilli is designated Region B, the intermediate zone of

Plate V. Amblyomma americanum adult

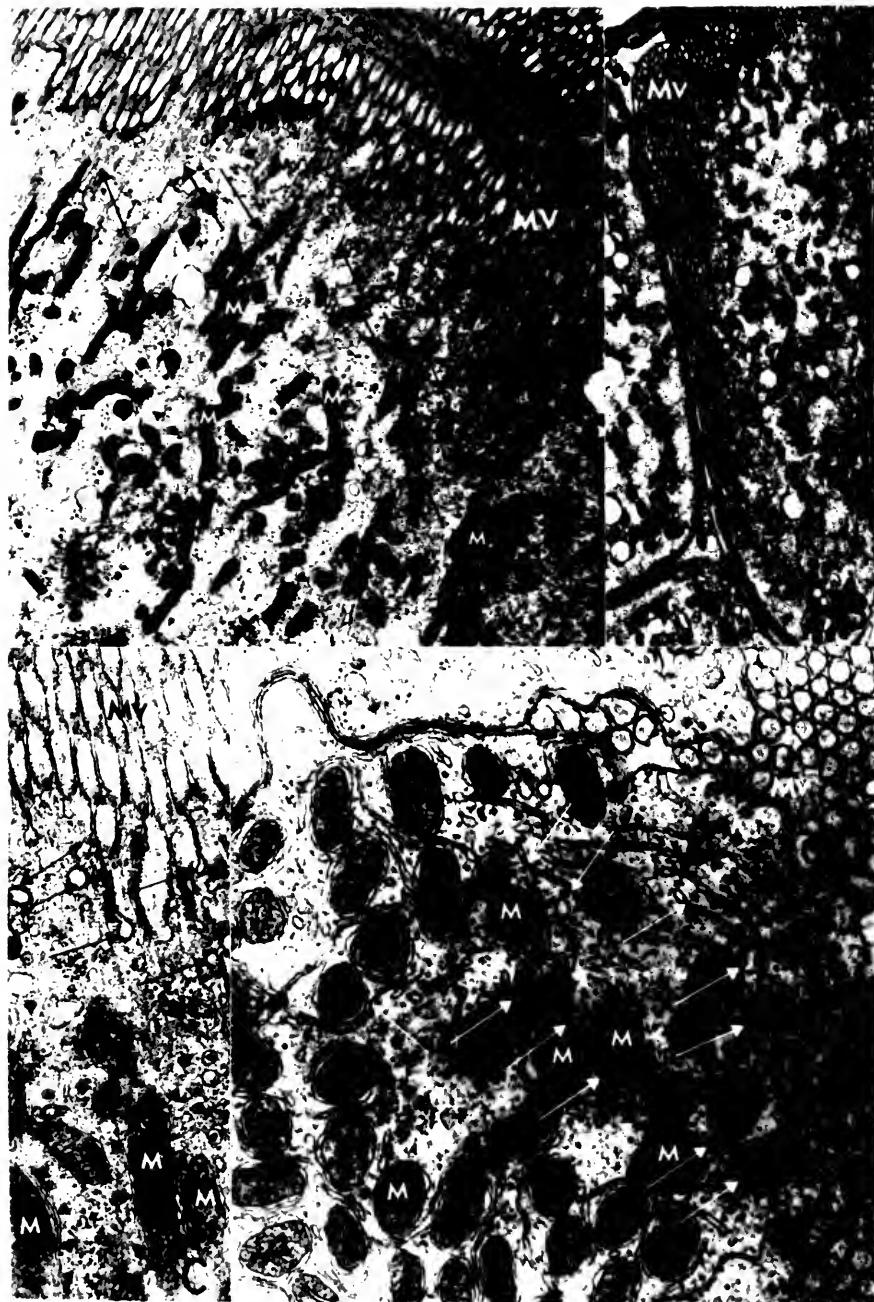
- A. Longitudinal section through tips of microvilli.
X22800

- B. Cross section of a peripheral portion of an eye. Axons (Ax) are surrounded by glial cell membranes, the mesaxons (Ma), glial cytoplasm (arrows), and an extracellular sheath (ES). Glial nuclei (GN) are located on the periphery of the eye. Cytoplasm of the axons and glial cells often contain electron opaque bodies (OB) and multi-vesicular bodies (MVB). X17100



Plate VI. Amblyomma americanum adult

- A. Oblique section of photoreceptor neuron through base of microvilli (Mv). Deep invaginations, intracellular channels (arrows), and numerous mitochondria (M) between membranes are characteristic of this zone (II). X11970
- B. Cross section of photoreceptor neuron through region B below microvilli (Mv). Numerous mitochondria, here in cross section, are characteristic of this region. X8740
- C. Oblique section of neuron at base of microvilli (M). Pinocytotic vesicles (arrows) form between microvillar bases. Mitochondria (M) lie in cytoplasm between intracellular channels. X15750
- D. Cross section of region B directly below microvilli (Mv). Mitochondria (M) are located between intracellular channels (arrows). X38000



cytoplasm. It is characterized by numerous elongate mitochondria, intracellular channels, and pinocytotic (or exocytotic) vesicles. The numerous sausage-shaped mitochondria (M) present in this region are associated with a system of intracellular channels (arrows (Plate VI A & D). Mitochondria are present in all parts of the neuron but are most prevalent in region B. These mitochondria lie between cytoplasmic sheets formed by intracellular membranous channels. Plate VI D is a photomicrograph of a cross section of this region and shows the mitochondria (M) between intracellular channels (arrows). The intracellular channels originate as inpocketings of the terminal membrane between the bases of the microvilli (Plate VI C). Vesicles (arrows) prevalent in this region appear to arise at the end of these membranous channels between the microvillar bases (Plate VI). This combination of microvilli, mitochondria and membrane-lined intracellular channels is very characteristic of transport cells such as Malpighian tubule cells, secretory, or glandular cells.

Region C. Nucleus-bearing portion of the soma

The nucleus of the photoreceptor neuron is the most prominent feature of zone C (Plate VII, VIII). Plate VII A & B are electron micrographs of cross sections through this region and show the relationship between the prominent nucleus (N) and the other organelles characteristic of the

Plate VII. Amblyomma americanum adult

- A. Cross section of photoreceptor neurons on periphery of eye just under the hypodermis (H). Two axons (Ax) and cross section of several neural soma, in zone C, showing nuclei (N) and rough endoplasmic reticulum (arrows). Glial nucleus (GN), mesaxons (Ma), and extracellular glial sheath (GS) cover the neurons. X5400
- B. Cross section of photoreceptor neurons in region C, the soma, at a higher magnification. The cytoplasm of the neuron in region C contains a prominent nucleus (N) and cisternae of rough endoplasmic reticulum (RER) but fewer mitochondria (white arrows) than region B. Note axons (Ax), glial nucleus (GN), mesaxons (Ma), and extracellular glial sheath (black arrows). X8740
- C. High magnification of glycogen-like (G) inclusions common in the cytoplasm of neural soma in region C. X57500
- D. High magnification of coated vesicles (CV) associated with Golgi complex in the cytoplasm around the nucleus. X57500
- E. Lower magnification of glycogen-like (G) and vesicular (CV) inclusions in cytoplasm of neural soma (region C). X43700



perikaryon. The nucleus is located in the center of the cell and is surrounded by conspicuous cisternae of endoplasmic reticulum (RER). Ribosomes are attached to the surface of the endoplasmic reticulum. The cisternae of this rough endoplasmic reticulum form concentric layers around the nucleus. The number of mitochondria in this zone is greatly reduced when compared to the preceding zone B (Plate VII B).

Two inclusions of similar size are common in the cytoplasm around the nucleus (Plate VII E). One type of inclusion appears to be glycogen (G) and the second, coated vesicles (CV). Plate VII C is an electron micrograph of alpha-glycogen rosettes present in the perikaryon in homogeneous masses (Plate VII E). Coated vesicles are elaborated by Golgi complexes and are present throughout the perikaryon in aggregates termed "Nebenkernen" (CV) by Fahrenbach in Limulus (Fahrenbach, 1970) and as isolated vesicles in the cytoplasm. One such "Nebenkern" (CV) is shown in Plate VII E. These coated vesicles (DV), shown at higher magnification in Plate VII D, are probably involved in the transport or storage of synthetic products. The amount of glycogen per cell is highly variable but the presence of coated vesicles in the photoreceptor neurons is fairly uniform.

Plate VIII. Amblyomma americanum adult

- A. Cross section of a single neuron throughout region C. Cisternae of rough endoplasmic reticulum (ERE) and Golgi (Go) are always found near the nucleus (N). Vesicular inclusions (arrows) are associated with the golgi. Other cytoplasmic inclusions include mitochondria (M) and multi-vesicular bodies (MVB). (Note longitudinal section of microvilli (M)). X17100

- B. Higher magnification of the same section showing the rough endoplasmic reticulum (RER), Golgi (Go), and associated vesicles. Two types of vesicles are formed by the golgi complex, small coated vesicles (arrows), and larger vacuolate vesicles (V). X28000

- C. Cross section of glial cell mesaxons (Ma), extra-cellular sheath (GS), bundles of fibrils (FB) often in glial sheath, and glial cell cytoplasm (arrows). X17100

- D. Cross section of glial cell sheath fibril bundles (arrows). X17100



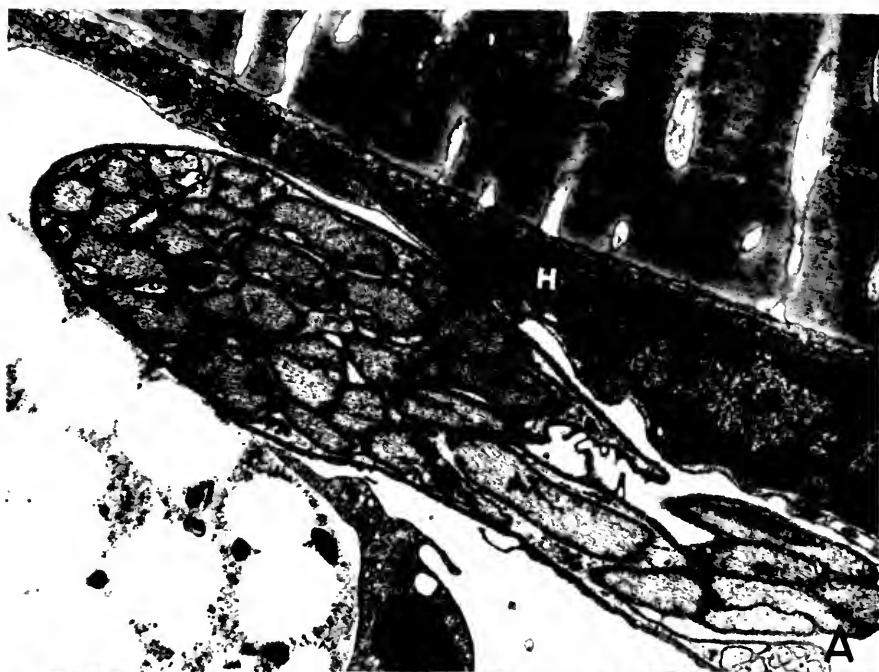
The relationship of the nucleus (N), rough endoplasmic reticulum (RER), Golgi (Go), and elaborated vesicles is shown in Plate VIII A & B. Cisternae of rough endoplasmic reticulum (RER) form concentric patterns around the nucleus and are generally the most conspicuous organelles of the perikaryon (Plate VIII A). Large numbers of coated vesicles (arrows) are discharged by the Golgi apparatus. The first, indicated by arrows in Plate VIII, are uniform in size and are "coated" by a layer of electron dense material. The second vesiculate type (V) are highly variable vaculoate vesicles that have smooth membranous walls apparently derived from the cisternal membrane of the golgi (Plate VIII B). The Golgi apparatus in the photoreceptor neurons generally consists of between 5-7 cisternae regardless of its size.

Region D. Axon

Each neuron attenuates rapidly behind the nucleus in the direction of the central nervous system to form a long axonal neurite that communicates directly with the optic lobes of the brain. The optic nerve of A. americanum larvae (Plate IX B & C, VII A) consists of 25-30 photoreceptor cell axons and the optic nerve of the nymph and adult contains 30-40 axons. Only one type of photoreceptor axon (Ax) is present in the optic nerve of A. americanum (Plate IX A & B). However, bundles of small axons (X) of unknown

Plate IX. Amblyomma americanum

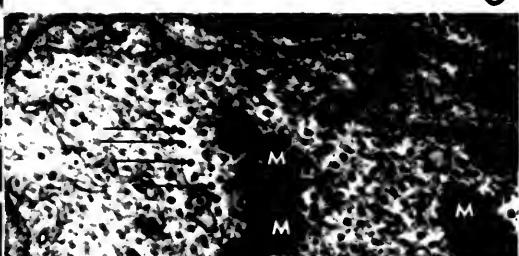
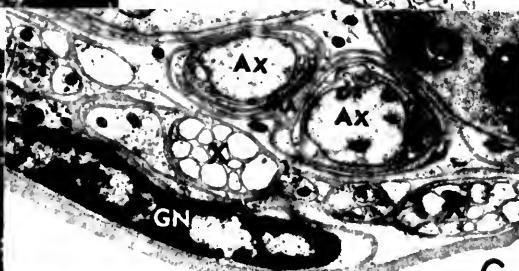
- A. Oblique section of larval optic nerve. Optic nerve contains 30-40 axons (Ax) from individual photoreceptor neurons. The optic nerve is located directly beneath the scutum (Sc) and scutellar hypodermis (H). X11970
- B. Cross section of 2 axons (Ax), a glial nucleus (GN), mesaxons (Ma), extracellular glial sheath (GS), and glial cell cytoplasm (arrows). X11500
- C. Cross section of axons (Ax), glial nucleus (GN), and mesaxonial (Ma) sheath surrounding the axons. Two bundles of smaller axons of unknown origin or function (X). X8740
- D. Cross section of a small portion of the axoplasm of a single axon showing characteristic microtubules (arrows) and mitochondria (M). X43700



A



C



M

function or origin were encountered in the optic nerve of adult ticks (Plate IX C). The optic nerve consists of photoreceptor axons, glial cells and a fibrous extracellular perineural sheath.

The axoplasm contains numerous longitudinally oriented microtubules (Plate IX arrows) that are orderly in their distribution. The axonal mitochondria are located more or less peripherally in the axoplasm adjacent to the axonal surface (Plate IX B & D). The axoplasm also contains infrequent dense opaque bodies (OB) (Plate V B, VIII D), possible residual or autophagic lysosomes. No other inclusions were encountered in the axonal region of the neuron.

The photoreceptor neurons are completely ensheathed by glial cells that insulate the neurons from the environment of the haemocoel (Plate VII A & B). The glial investment of Amblyomma is intermediate between the condition encountered in the myelinated and unmyelinated nerves of vertebrates. The glial cytoplasm of myelinated nerves is obliterated leaving a glial membranous sheath termed myelin. In Amblyomma and other arthropods glial cytoplasm and glial cell membrane cover the axon. This condition is common in the arthropods and nerves of this type are called tunicated nerves. In electron micrograph V B the glial cytoplasm (arrows) can be seen between the glial cell membranes (Ma). The neurons lie within invaginations of the glial cells.

These invaginations of the glial cell membrane form long double membrane mesaxons (Ma) that wind around the axons and neural cell somata (Plate XI C, VII B). Each neuron is generally surrounded by two or three mesaxonal membranes. The mesaxons often bifurcate (Plate VII B) and encompass several axons. Glial cytoplasm contains few organelles or inclusions but mitochondria, multivesiculate bodies, and opaque inclusions are sometimes present (Plate V B). Glial cell nuclei (GN) are located on the periphery of the neurons (Plate IX B, C; Plate V B) and conform to the outline of the axons.

A fibrous extracellular perineural sheath (GS) covers the glial cells, axons, and photoreceptor neurons (Plate IX B, VII B). Embedded in the glial sheath, termed the neuralemma by some authors, are bundles of fibrils (Plate VIII C & D). These fibril bundles are not encountered in a predictable manner. In Plate VII B the glial sheath does not contain any fibrils. Plate VIII C and Plate VIII D are sections of these fibril bundles sectioned obliquely and in cross section respectively. The extracellular glial sheath is composed of an amorphous material very similar in appearance to the basement membrane (compare BM and GS in VII A).

The optic nerve, upon leaving the eye, is located directly beneath the scutellar hypodermis (H) and remains in close proximity to the hypodermis until it enters the brain laterally.

Discussion

Horridge (1965) stated that the eyes of ticks are "aberrant" and "improbable" as "efficient sense organs." The eyes of ticks do not appear aberrant or improbable as sense organs when studied microanatomically with the electron microscope. The eyes of Amblyomma americanum must be considered in the context of all rhabdomeric photoreceptors, particularly those of the Mollusca and the Platyhelminthes, and not just the highly advanced arthropod compound eyes. They differ anatomically from any of the other arthropod eyes that have been studied to date. The principal difference is their simplicity. Despite this simplicity, the eyes of Amblyomma possess all the structures necessary to make them fully functional photoreceptors. The eyes have a lens, well-developed photoreceptor neurons, and an optic nerve that communicates directly with the optic lobes of the brain. The microanatomy of the neural cells beneath the lens identifies them with little doubt as photoreceptor cells. Electron microscopy was necessary to visualize the structures, in particular the microvilli which are characteristic of rhabdomeric photoreceptors of light microscopy.

The most unusual aspect of the tick eye is the corneal lens. Bonnet (1907) described perpendicular striae accentuated by black pigments in the lens and Gossel (1935, Fig. 36)

noted the chitin of the lens is pierced by fine "canals." The "striae" they described were probably the bundles of lenticular pore canals described in this study (Plate III A). These bundles of pore canals appear as bright streaks when thick sections are viewed with the light microscope. This light-conducting property of the pore canal bundles indicates that they may act as light or wave guides. The lenses of all other arthropod eyes studied are devoid of pore canals or other structures and the lens of Amblyomma is the first described with such structures. In Limulus the unmodified cuticle between the corneal facets of the compound eye has pore canals (Fahrenbach, 1969) and may represent an intermediate condition between the lens of Amblyomma and the typical ommatidial facet or unicorneal lens in the chelicerates.

The pore canals of insects have been studied in detail (Locke, 1959, 1961) as have the pore canals in the integument of ticks (Nathanson, 1967; Beadle, 1972) and mites (Wharton et al., 1968). The pore canal bundles in the lens of Amblyomma have not been described from any other arthropods and represent a completely different adaptation of cuticular pore canals in the arthropods. The pore canals follow a curved path through the lenticular cuticle and converge on a point directly above the photoreceptor neurons. The diameter of the pore canal bundles becomes progressively smaller and the pore canals converge as they approach the

inner surface of the lens. If the pore canal bundles actually function as light or wave guides their convergence and reduction in diameter may serve to intensify the light impinging on the photoreceptor neurons. Light energy gathered on the outer surface of the lens where the surface area is greatest would be condensed and concentrated on the smaller inner lenticular surface over the photoreceptor neurons.

The lenses of other chelicerate eyes are strongly biconvex (Horridge, 1965; Curtis, 1970; Eakin and Brandenberger, 1971; Melamed and Trujillo-Cenoz, 1966; Fahrenbach, 1963). The lenses of ticks described with light microscopy are simple convex or very slightly biconvex (Gosse, 1935). The lens of Amblyomma americanum is slightly biconvex. It has an off-center bulge on the internal ventral surface of the lens.

Region A. Microvilli

All photoreceptors, rhabdomeric and ciliary, have parallel membranous arrays. Arrays of membranes are characteristic of all photoreceptors and it is assumed that their function is to provide an ordered plano-arrangement of membrane-bound photochemicals (Eakin, 1965, 1968). Two lines of evolution are apparent in the evolution of multiple membrane systems, the ciliary line and the rhabdomeric line (Eakin, 1965, 1968; Varela, 1971). The rhabdomeric line,

characterized by the microvillus, is believed to have arisen as an early offshoot of the ciliary line and developed independently (Eakin, 1965). Varela (1971) postulates that the molluscs and arthropods do not belong in the same evolutionary line and suggests that the Mollusca constitute a third line of evolution.

The microvilli of the photoreceptor neurons of Amblyomma americanum are typical of those encountered in other arthropod photoreceptors. One important aspect of the microvilli is the absence of any microvillar interaction. The microvilli are closely packed (Plate IV A, B; V A) without the development of a highly ordered hexagonal "honeycomb" relationship characteristic of the insects such as the cockroach (Smith, 1968), dipterans (Trujillo-Cenoz, 1972; Wolken, 1971), beetle (Meyer-Rochow, 1973), hemipterans (Burton & Stockhammer, 1969), ants (Wolken, 1971), and many crustacea (Eguchi & Waterman, 1966; Wolken, 1971) or the formation of tight junctions between adjacent microvilli as in Limulus (Fahrenbach, 1969; Lasansky, 1967; Nolte & Brown, 1971), Lycosa (Melamed & Trujillo-Cenoz, 1966); Phalangids (Curtis, 1970), Octopus (Moody & Robertson, 1960), and the larval mosquito eye (White, 1967). The microvilli are completely free within the glial sheath and tight packing of the microvilli of Amblyomma americanum produces neither the hexagonal honeycomb configuration nor occluded intercellular bridges (Plate IV B). Tight

packing of microvilli in the eye of mandibulate arthropods (Crustacea and Insecta) produces hexagonal packing with a uniform intervillar space of constant dimensions. Tight packing of microvilli in chelicerate eyes produces an occluded intervillar space with tight junctions at points where adjacent microvilli touch. In this respect the photoreceptor microvilli of Amblyomma americanum show affinities with the microvilli borne on the photoreceptor neurons of planarians (Röhlich & Török, 1961; MacRae, 1964) and snails (Röhlich & Török, 1963) whose microvilli exhibit neither occluded intervillar space or hexagonal packing. Hexagonal packing and occlusion of the intervillar space will be considered in this study as advanced evolutionary characteristics. Free microvilli as encountered in the ticks, planarians, and snails are considered primitive.

The longitudinal planes of the microvilli of several neurons in Plate II A and B are perpendicular. This orthogonal arrangement of microvilli provides the anatomical basis of polarized light perception (Waterman & Horch, 1966). This perpendicular or orthogonal pattern was encountered only in larval ticks and not in nymphal or adult ticks. This aspect of the tick eye deserves further investigation to determine if the eye functions as a polarizer.

Region B. Intermediate zone of intracellular channels

The system of membrane-bound intracellular channels and vesicles originating at the base of the microvilli is very characteristic of rhabdomeric photoreceptor neurons. A system of subrhabdomeric cisternae and vesicles occurs in Limulus (Fahrenbach, 1968), Lycosa (Melamed & Trujillo-Cenoz, 1966) 7 species of the phalangids (Curtis, 1970), 5 species of decapod crustaceans (Eguchi & Waterman, 1966, 1968), Drosophila (Waddington & Perry, 1960), the toad bug, Gelastocoris (Burton & Stockhammer, 1969), a mosquito larva (White, 1967), 4 genera of dipterans (Trujillo-Cenoz, 1965a, 1972), snail Helix (Röhlich & Török, 1963), and 3 species of turbellarian platyhelminthes (Röhlich & Török, 1961; MacRae, 1964). These are common structures associated with rhabdomeric photoreceptor neurons and are found in all the eyes studied to date. The presence of these organelles in the submicrovillar portion of the photoreceptor neurons of Amblyomma americanum adds credence to their identification as retinular cells. Long oval mitochondria are oriented longitudinally and occur in large numbers between the membrane-lined channels. Mitochondria are present throughout the neural cell body and axoplasm but are very abundant in region B (Plate VI A, B, & D) and rather sparse in other zones (Plate VII A & B). The large numbers of mitochondria located in this region indicate an intense rate of metabolic activity and energy utilization.

Most authors interpret the presence of membranous intracellular channels, associated mitochondria, and numerous vesicles in this region as indicating protein uptake by pinocytosis (Waddington & Perry, 1960; Melamed & Trujillo-Cenoz, 1966, 1968; Burton & Stockhammer, 1969; Curtis, 1970). An alternate interpretation was provided by Fahrenbach (1968). He believes the coated and vacuolate vesicles are part of a secretory sequence originating with the rough endoplasmic reticulum and Golgi that ends with secretion into the rhabdom of the eye. Whether this region represents an area of pinocytotic uptake or exocytotic secretion will require further study. The large number of mitochondria (energy utilization) indicates pinocytosis but no known material is present in the rhabdom other than haemolymph. The microvilli of the tick eye are separated from the haemocoel by their glial investiture only and materials present in the haemocoelic fluid would be more available than in the rhabdomate eyes.

The photoreceptor neurons of Amblyomma americanum have several important features in common with other rhabdomeric eyes. These shared features include terminal microvilli perpendicular to the light path, similar cytoplasmic organelles and organization, and an axonal neurite that communicates with the optic lobes of the brain. The important differences that separate it from other arthropod photoreceptors are terminal microvilli oriented in

longitudinal axis of the neuron, the arhabdomate eye, the unusual construction of the lens, and the total absence of pigment in the neurons or associated cells.

The eyes of Amblyomma are the first arhabdomate or arhabdomeric eyes described in the phylum Arthropoda. The unicorneal eyes of chelicerates and the compound eyes of the mandibulates are very similar in structure with the exception of the lenticular structures. The retina of chelicerates (Melamed & Trujillo-Cenoz, 1966; Curtis, 1970; Eakin & Brandenberger, 1971) is composed of repeating units very similar in structure and organization to the ommatidia in the mandibulates. No such structures are present in the arhabdomate eyes of Amblyomma. This arhabdomate condition is not shared with the only other acarine eye to be studied, Tetranychus urticae (Mills, 1973), that has both rhabdomeres and rhabdoms.

The simplicity of the Amblyomma eye raises the question of the secondary reduction. Is the eye of Amblyomma primitive, possibly an archetype of arthropod eyes, or is it secondarily reduced? I believe the eye to be very primitive and not the product of secondary reduction. It could very easily be an archetype of the arthropod eye since more advanced retinular cell types could be derived from it by merely changing the shape of the neuron. The tick eyes represent a very plausible step in the evolution of the compound eye of the mandibulates and unicorneal eye of the

chelicerates. It is an evolutionary step never before recognized.

Two important considerations support this view of simplicity rather than secondary reduction. First, the cytoplasmic organelles, cell structures, and organization are not reduced but are well developed. Organelles are numerous and characteristic of a very generalized cell. Advanced arthropodan photoreceptors have retinula cells that appear metabolically inactive when compared with the photoreceptor neurons of Amblyomma (see Chapter II of this study; Trujillo-Cenoz, 1972; Boschek, 1971). Second, the tick eyes exhibit important microanatomical affinities with the eyes of two phylogenetically lower animals, the snails (*Mollusca*) and flatworms (*Platyhelminthes*). The photoreceptor neurons of all three (snails, flatworms, and ticks) are strikingly similar. They all possess terminal microvilli parallel to the longitudinal axis of the photoreceptor neurons, similar organelles and cellular organization. The neurons are all parallel and the microvilli are all oriented in the same direction. In more advanced eyes the retinular cells are located around a central rhabdom and the microvilli are oriented in from two to eight different directions. No microvillar tight junctions or highly ordered hexagonal honeycomb pattern have developed in the snails, flatworms, or ticks as a result of tight packing of microvilli. The most advanced rhabdometric eye, that of Octopus, has

microvilli joined by tight junctional bridges (Moody & Robertson, 1960). The microvilli of flatworms, snails, and ticks have the assumed primitive condition of no intervillar interaction.

The archetypal eye of arthropodan stock was possibly very similar to the eye of ticks. This primitive condition probably persisted in the ticks primarily due to their use of tarsal sensilli as the primary sensory receptors. The mites have well-developed tarsal sensilli and in the ticks these sensilli are of particular importance in host location. Dependence on tarsal receptors for host location could account for maintenance of the primitive condition in tick eyes.

Varela (1971) postulates a separate rhabdomic evolutionary line for the Mollusca. The obvious affinities of the Platyhelminthes, Mollusca, and tick eye place them in the same evolutionary line of sensory receptors.

CHAPTER II

THE MICROANATOMY OF THE EYE OF THE "SCREWWORM FLY," Cochliomyia hominivorax (Coquerel)

Introduction

The eradication of the screwworm fly, Cochliomyia hominivorax (Coquerel), from Florida is one of the most successful control programs instituted against a major pest insect resulting in an estimated 14 million dollars a year saving to cattlemen (Cromroy, 1971). The larva of the screwworm fly is an obligate parasite and eats only the living flesh of warm-blooded vertebrates.

The control program involved breaking the life cycle of the fly by introducing overwhelming numbers of sterile male flies into an area to mate with native fertile females. The program was successful in Florida and eliminated the fly from most of the southeastern U.S. after its initiation. The sterile male technique has been used in the southwestern United States along the Mexican border to prevent the usual northward spread of the fly each year. A projected program is now underway to use the sterile male technique in Mexico in hopes of eliminating the fly from all of Mexico and prevent its reintroduction into the United States.

The success of the sterile male technique depends upon the production of large numbers of sterile male flies that are competitive with wild flies. In order to be fully competitive in the wild, they must be so close to their wild counterpart behaviorally and physically that the female flies will mate with them readily. Mass rearing of the insects is therefore very important to the success of this program. In all attempts of mass rearing certain dietary and genetic problems are encountered because of the requirements of the rearing program. Mass rearing tends to cause either genetic deterioration of the breeding stock used or poor quality flies due to inadequacies in the larval diet.

One of the problems facing the screwworm project is the lack of biological information about this fly. This study was initiated to produce information on the microanatomy of the eye of the adult fly. Irradiated and unirradiated flies were used to study the possible effect of irradiation on the eye of the fly. Several closely related genera have been studied extensively with electron microscopy and this study was also undertaken to confirm these prior studies and expand the information available on the eyes of this important group of flies.

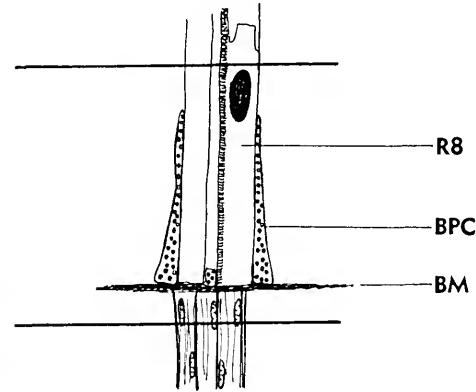
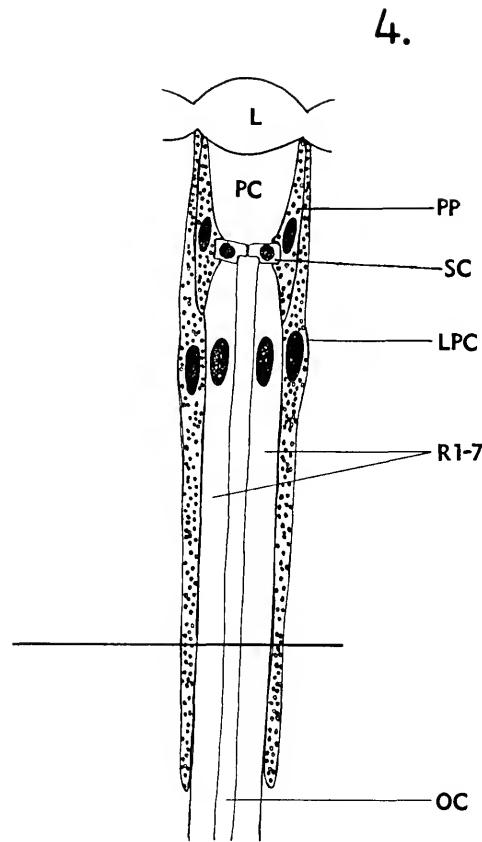
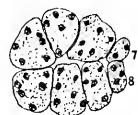
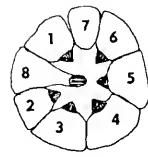
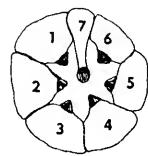
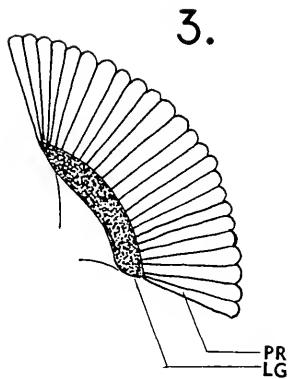
Literature Review

Most adult insects have 2 large compound eyes located on the lateral margins of the head. In general the compound eyes of all insects are very similar. They all possess a peripheral retina composed of repeating units called ommatidia. Each ommatidium consists of the image-forming dioptric apparatus and a variable number of unipolar photoreceptor neurons, the retinular cells. The retinular cells possess rhabdomeres composed of parallel arrays of thousands of microvilli (Eakin, 1965). Photopigment molecules are presumably located on the inner surface of the microvillar membrane. The rhabdomeres within a single ommatidium form a central fused rhabdom in most arthropods (Trujillo-Cenoz, 1972). Dipterans of the suborder Cyclorrhapha have rhabdomeres that project into a central extracellular ommatidial space. This arrangement is termed an open rhabdom. In addition the dioptric apparatus of dipterans differs anatomically in that it has a pseudocone beneath the cornea (Trujillo-Cenoz, 1972; Bernhardt *et al.*, 1972). These anatomical differences combined with the quantity and advanced nature of the research on dipteran eyes make it appropriate to deal with it as an isolated unit.

Seven genera of cyclorrhaphan Diptera have been studied in detail using electron microscopy: Musca domestica L. (Boschek, 1971, 1972; Braitenberg, 1967, 1972; Kirschfeld,

Figure 3. Schematic diagram of Cochliomyia hominivorax eye showing the relative position of the peripheral retina (PR) and lamina ganglionaris (LG).

Figure 4. Schematic diagram of a longitudinal section of an ommatidium of the compound eye of Cochliomyia hominivorax. BM, basement membrane; BPC, basal pigment cell; L, lens; LPC, large pigment cell; OC, ommatidial cavity; PC, pseudocone; PP, primary pigment cell; R1-7, retinula cells 1-7; R8, inferior retinula cell.



1967, 1972; Kirschfeld & Francheschini, 1968, 1969; Kirschfeld & Reichardt, 1970; Campos-Ortega & Strausfeld, 1972), Drosophila melanogaster (Dannel, 1957; Fuge, 1967; Waddington & Perry, 1960), Anastrepha suspensa (Loew) (Agee, in prep.), the following 3 genera that were studied together as a unit Chrysomia sp., Lucilia sp., and Sarcophaga sp. (Melamed & Trujillo-Cenoz, 1968; Trujillo-Cenoz, 1965a, 1965b, 1969; Trujillo-Cenoz & Melamed, 1962, 1963, 1966a), and Sympycnus lineatus Loew (Trujillo-Cenoz & Bernard, 1972). The eyes of these flies are structurally very similar and I will review the structures common to the 7 genera and introduce the terminology to be used in this study.

The dioptric apparatus is composed of a corneal lens and an underlying gelatinous crystalline body termed the pseudocone (Trujillo-Cenoz, 1972). The pseudocone is a soft amorphous substance enclosed in a cup-shaped cavity formed by 2 cells called the primary pigment cells by Boschek (1971). The pseudocone is extracellular, contains no inclusions or cellular organelles (Trujillo-Cenoz & Melamed, 1966a) and has approximately the same refractive index as the vitreous humor of the human eye (Bernhardt et al., 1972). The proximal end of the pseudocone cavity is closed by 4 wedge-shaped cells forming a plate-like floor of the cavity (Trujillo-Cenoz, 1965a). Extracellular amorphous prolongations of the rhabdomeres extend into an

invagination of the proximal membrane of the Semper cells (Trujillo-Cenoz, 1965a, 1972). Boschek (1971) termed these rhabdomeric prolongations, the rhabdomere caps. Boschek (1971) and Trujillo-Cenoz (1965a, 1972) postulate that the function of the Semper cells is to provide mechanical and optical coupling between the dioptric apparatus and the open rhabdom.

Three types of pigment cells are present in the ommatidia: (1) the primary pigment cells, (2) the large pigment cells located distally and containing a purple pigment (Trujillo-Cenoz, 1972), and (3) small basal pigment "cells" near the basement membrane of the peripheral retina that contain a yellow-brown pigment (Trujillo-Cenoz, 1972). The basal pigment "cells" in Aedes egyptii (L.) are actually not cells but pigment filled bags at the end of thread-like processes of the Semper cells (Brammer, 1970). Similar Semper cell processes have been found in Musca (Boschek, 1971) but are not known to connect to the 4 basal pigment cells.

Eight photoreceptor cells, the retinula cells (R1-R8), make up the photosensitive portion of the ommatidium (Trujillo-Cenoz, 1965a; Melamed & Trujillo-Cenoz, 1968; Boschek, 1971; Trujillo-Cenoz & Bernard, 1972). Six of these, R1-R6, have rhabdomeres that are peripherally located around the extracellular space forming the central ommatidial cavity.

The centrally located seventh rhabdomere consists of the rhabdomeres of retinular cells R7 and R8. The rhabdomere of R7, termed the superior central cell (SCC), forms the distad portion of the central rhabdomere. The rhabdomere of R8, termed the inferior central cell (ICC), forms the proximad portion of the central rhabdomere. Rhabdomeres R7 and R8 are subequal, the superior rhabdomere (R7) is long and the inferior (R8) is relatively short. The axons of R7-R8 do not synapse in the first visual ganglion, the lamina, but pass directly into the second, the medulla (Melamed and Trujillo-Cenoz, 1968; Trujillo-Cenoz, 1972). The rhabdomere of R7-R8 differs from R1-R6 by being smaller in diameter and cylindrical rather than a truncated cone (Boschek, 1971).

The rhabdomeres are composed of tightly packed microvilli. The orientation of microvilli in rhabdomeres R1-R6 is such that the microvilli of the following are parallel: R1 and R4, R2 and R5, and R3 and R6 (Boschek, 1971; Melamed & Trujillo-Cenoz, 1968). The orientation of the rhabdomeric microvilli in the central cells (R7 and R8) is orthogonal in Musca, Crysomyia, Lucilia, and Sarcophaga (Boschek, 1971; Melamed & Trujillo-Cenoz, 1968; Trujillo-Cenoz, 1972; Bernhardt et al., 1972; Trujillo-Cenoz & Bernard, 1972).

In the species, Sympycnus lineatus, two types of ommatidia are present. Half the ommatidia, those with yellow

corneal facets, have the usual orthogonal or perpendicular arrangement of the central rhabdomeric microvilli. In the remaining half of the ommatidia, those with red corneal facets, the microvilli, are parallel to one another (Trujillo-Cenoz & Bernard, 1972).

The orthogonal arrangement of microvilli has been postulated as a two-channel analyzer of plane-polarized light (Waterman & Horch, 1966; Melamed & Trujillo-Cenoz, 1968; Trujillo-Cenoz, 1972). Rhabdomeres with parallel arrangement of microvilli are postulated to diminish the absorption of plane-polarized light in the opposite or perpendicular plane (Trujillo-Cenoz & Bernard, 1972; Trujillo-Cenoz, 1972). The parallel microvilli are oriented in the vertical plane and are believed to minimize the absorption of horizontally polarized light, i.e., reflected light or "glare." A similar arrangement is found in the ventral portion of the eye of the water strider, Gerris sp. (Schneider & Langer, 1969), and is believed to allow a better view into the water by differential screening of surface reflected light.

Directly beneath the peripheral retina is the first synaptic field of the eye, the lamina ganglionaris. The lamina is divided anatomically into three layers: the external fenestrated layer, an intermediate layer of unipolar cell soma, and the proximal plexiform layer (Trujillo-Cenoz, 1965b; Boschek, 1971). The fenestrated layer

contains tracheoblasts, trachea, and bundles of eight retinular cell axons, the pseudocartridges. The somata of unipolar second order neurons are located in the intermediate or unipolar cell soma layer. The plexiform layer has two second order axons termed L1 and L2 by Braitenberg (1967) which synapse with the axonal fibers from retinular cells R1-R6 (Trujillo-Cenoz, 1965b) to form the optical cartridges. The optical cartridges are surrounded by epithelial cells that make intimate contact with retinular axons R1-R6 by means of specialized glial projections called capitate projections (Trujillo-Cenoz, 1965b; Boschek, 1971). These structures were first believed to be synaptic in nature and were termed synaptic buttons by Pedler and Goodland (1965). The true synaptic loci however are formed by T-shaped synaptic ribbons (Trujillo-Cenoz, 1965a, 1965b; Boschek, 1971, 1972).

Methods and Materials

The flies used in this study were obtained from two sources. Unirradiated flies were reared by Dr. Gerald Holt, USDA, APHIS, Fargo, North Dakota. These flies were fixed and embedded in Fargo by Dr. Holt's laboratory personnel following the same preparative technique used on irradiated flies. Irradiated flies were reared in Mission, Texas, by USDA, APHIS in their rearing facility and shipped via air mail to Gainesville as pupae. The pupae were placed in a

shallow cup in a holding cage consisting of an aluminum frame with tube gauze stretched over it. Cotton saturated with a mixture of honey and water was provided as a source of sugar and water for the adult flies. Flies from 3 to 6 days of age were utilized.

Living flies were submerged in paraformaldehyde-glutaraldehyde fixative (Karnovsky, 1965) for dissection. Following removal and bisection, the eyes were transferred to fresh fixative for 4 hours at room temperature, rinsed in 0.1 M cacodylate buffer (pH 7.2) for 20 minutes and post-fixed in 2% osmium tetroxide for 20-24 hours at 4°C. Rapid dehydration at intervals of 5 minutes in 25, 50, and 75% ethanol followed a second rinse in 0.1 M cacodylate buffer. The eyes were held in 2% uranyl acetate in 75% ethanol for 4 hours at 4°C. Dehydration was completed with 5-minute changes of 95, 100% ethanol and two changes of 100% acetone at room temperature. The eyes were infiltrated for 1 hour with 50% Spurrs plastic (Spurr, 1969) and 24 hours in 100% plastic prior to polymerization at 60°C for 24 hours.

Silver and light gold sections were cut using a duPont diamond knife on a Porter-Blum-MT-2 ultramicrotome, picked up on 75-mesh copper grids covered with a Formvar film and post-stained with uranyl acetate and lead citrate (Reynolds, 1963) prior to examination with either a Hitachi HU11C or HU11E electron microscope at 75 kV.

Results

The Peripheral Retina

The peripheral retina of Cochliomyia hominivorax consists of hexagonally packed ommatidia (Fig. 4). Each ommatidium has a dioptric apparatus and 8 photoreceptor cells, the retinular cells (R1-R8). The diptric apparatus is composed of a corneal lens (L) and a gelatinous pseudocone (PC). Surrounding the pseudocone and forming the lateral walls of the pseudocone cavity are the primary pigment cells (PP). The floor of the pseudocone cavity is formed by a rectangular plate of wedge-shaped Semper cells (S). In addition to the primary pigment cells, two other types of pigment cells are found in the peripheral retina: the large pigment cells (LPC) located laterally and the basal pigment cells (BPC).

The receptor region of the peripheral retina is composed of the 8 photoreceptor or retinula cells. Six of these retinular cells (R1-R6) are located peripherally around the central ommatidial cavity (OC). Retinular cells, R1-R6, bear independent rhabdomeres made up of microvilli. The rhabdomeres of retinular cells, R7 and R8, are centrally located in the ommatidial cavity and form a single central rhabdomere. The distal portion is formed by the rhabdomere of R7, termed the superior central cell, and the proximal

portion by R8, the inferior central cell. The two central retinular cells are subequal in length, R7 (SCC) is longer being approximately 170 microns in length and R8 (ICC) is approximately 60 microns in length.

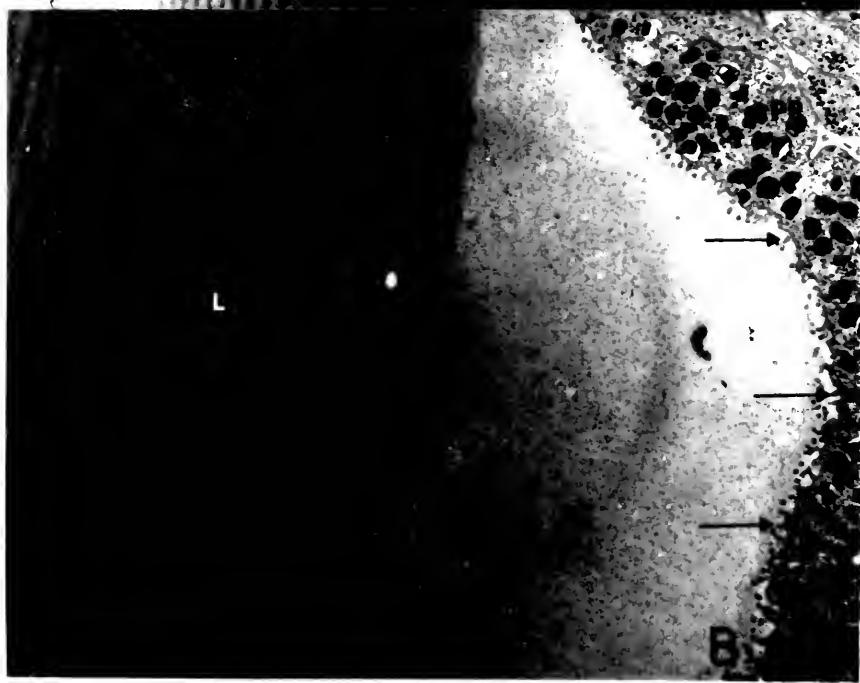
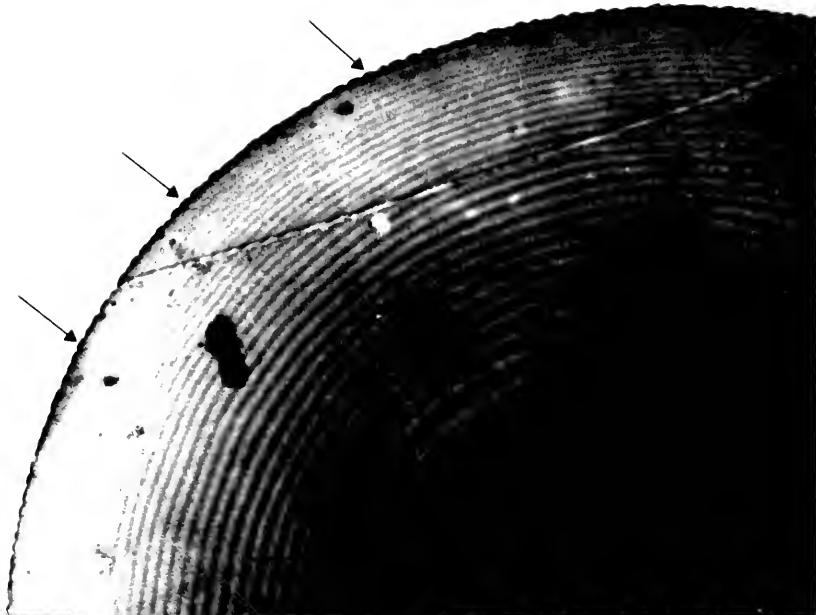
Each retinular cell has an array of microvilli that extends from the distal to the proximal end of the cell body. The blind ends of the microvilli project into the ommatidial cavity. The microvilli borne by one cell are termed a rhabdomere and the rhabdomeres whether fused or separate are termed a rhabdom. In most arthropods the rhabdomeres are fused into a central rhabdom. Cochliomyia hominivorax and the other members of the suborder Cyclorrhapha have an ommatidial space or cavity (OC) that separates the rhabdomeres and extends the length of the ommatidium. This configuration is termed an open rhabdom.

The Dioptric Apparatus

The dioptric apparatus of the adult screwworm fly consists of a corneal lens and an amorphous gelatinous pseudo-cone. The lens (Plate X A) is a modification of the cuticle. It has no pore canals or other structures usually associated with the cuticle of insects. Plate X A is a cross section of a lens showing the alternating "dense" and "rare" bands believed by several authors to act as interference filters (Trujillo-Cenoz, 1972; Bernard & Miller, 1968; Bernard

Plate X. Cochliomyia hominivorax unirradiated

- A. Cross section of lens showing alternating dense and rare bands believed to act as interference filters. Note small round protuberances on surface of lens (arrows). X8740
- B. Off center longitudinal section of lens (L) and pseudocone (PC). Primary pigment cells IPP form the lateral walls of the pseudocone cavity. Note microvilli-like projections of primary pigment cell membrane (arrows) and alternating dense and rare bands.



et al., 1972). Plate X B shows these bands in longitudinal section.

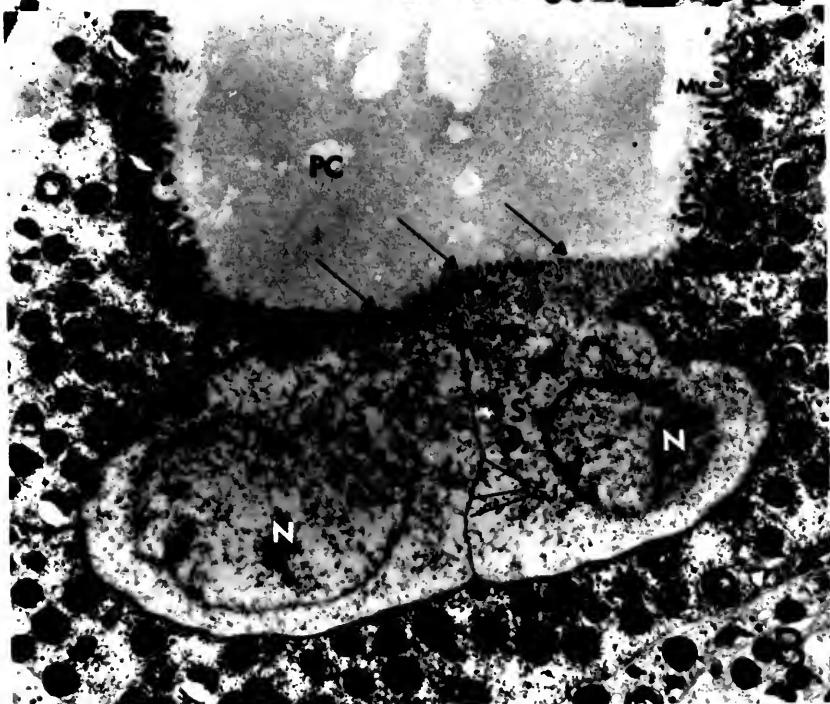
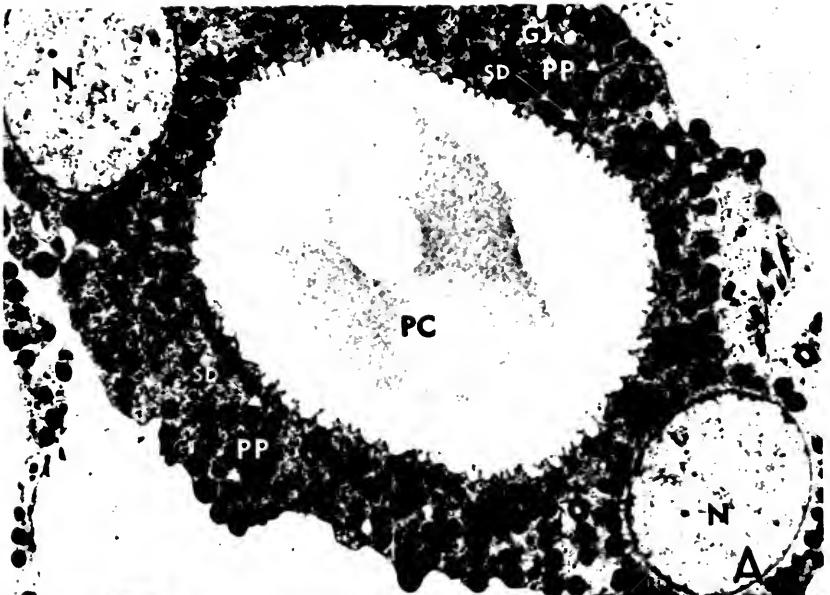
Beneath the corneal lens is the pseudocone cavity containing the extracellular amorphous pseudocone (PC) (Plate X B). The pseudocone cavity is formed by the primary pigment cells (PP) (Plate X B, XI A & B). There are two primary pigment cells that form the lateral walls of the pseudocone cavity in Cochliomyia hominovorax (Plate X B; XI A & B). Numerous irregular microvillar-type (Mv) evaginations of the primary pigment cell membrane project into the pseudocone cavity (Plate XI B). The pseudocone is not completely homogeneous and contains material of greater electron density irregularly concentrated toward the center of the pseudocone (Plate XI A). Plate XI A is a micrograph of a cross section through the pseudocone (PC), the primary pigment cells (PP), and the primary pigment cell nuclei (N). The primary pigment cells are tightly bound by spot desmosomes (SD), at the edge of the cell that bounds the pseudocone, and gap junctions (GJ) over the rest of the adjoining membrane.

The Semper Cells

The proximal end of the pseudocone cavity is closed by 4 flattened wedge-shaped cells that form a rectangular plate-like floor (Plate XIII B, XI B). Plate XI B shows an off-center longitudinal section (Fig. 5) of the pseudocone (PC)

Plate XI. Cochliomyia hominivorax unirradiated

- A. Cross section of pseudocone (PC) and primary pigment cells (PP). Two primary pigment cell nuclei (N) and pigment filled vacuoles are most prominent organelles in the primary pigment cells. Note gap junction (GJ) and spot desmosome (SD) cell to cell contact between primary pigment cells. X8740
- B. Off-center longitudinal section of pseudocone (PC) and Semper cells (S). Semper cells have short unequal "microvilli" (black arrows) that project into the pseudoncone cavity. Note Semper cell nuclei (N), gap junction (GJ), and spot desmosome (SD). X11970



and the Semper cells (S). Plate XI B is an electronmicrograph of a cross section of the Semper cell plate. Short irregular projections (arrows) of the Semper cells' distal membrane project into the pseudocone cavity (PC) (Plate XI B).

The Semper cells are joined by cell junctions that are very similar in structure to those of the primary pigment cells. Plate XI B shows the cell junction between 2 Semper cells (S). Their membranes are joined near the pseudocone cavity by a spot desmosome (SD) or macula adherens and the remaining membrane is joined by a gap junction (GJ) or zonula occludens (Plate XI B). Spot desmosomes are cell contacts that involve thickening of the cytoplasmic surface of the cell membrane and gap junctions are cell contacts with a partial obliteration of the intercellular space (Satir & Gilula, 1973). The spot desmosome (SD) and gap junction (GJ) between the Semper cells provide a close sealed apposition and seal the bottom of the pseudocone cavity.

The distal membrane of the Semper cells is produced into a network of ridges that project into the pseudocone cavity. In cross section (Plate XI B) these ridges appear to be irregular microvillar-type projections. Plate XII shows two magnifications of the junction of the Semper cells and the pseudocone. This network of Semper cell membrane ridges projects into the pseudocone cavity presumably holding the gel-like pseudocone in place.

Plate XII. Cochliomyia hominivorax unirradiated

- A. Cross section through a junction of the pseudocone (PC), Semper cells (SC), and primary pigment cells (PP). Projections from the Semper cells into the Pseudocone cavity form a network (arrows). Note spot desmosome (SD) joining Semper cell membranes. X5890
- B. Higher magnification of the same cross section of a junction between pseudocone (PC) and Semper cells (SC). X38000



Plate XIII. Cochliomyia hominivorax unirradiated

- A. Cross section of an ommatidium near junction of the pseudocone (PC) and 4 Semper cells (S). Note presence of primary pigment cells (PP), primary pigment cell nucleus (N), large pigment cells (LPC), and distal end of the ommatidial cavity (OC). Numerous granular inclusions (arrows) present in the cytoplasm of the large pigment cells and make it possible to distinguish them from the primary pigment cells. Spot desmosomes joining distal membranes of Semper cells are indicated by arrows. X6650
- B. Cross section of the 4 Semper cells (S) showing the rhabdomere caps (RC) and the ommatidial cavity (OC). Four Semper cell nuclei (SN) and 2 large pigment cell nuclei (PN) are shown. Note that only gap junction (GJ) present.
X7600

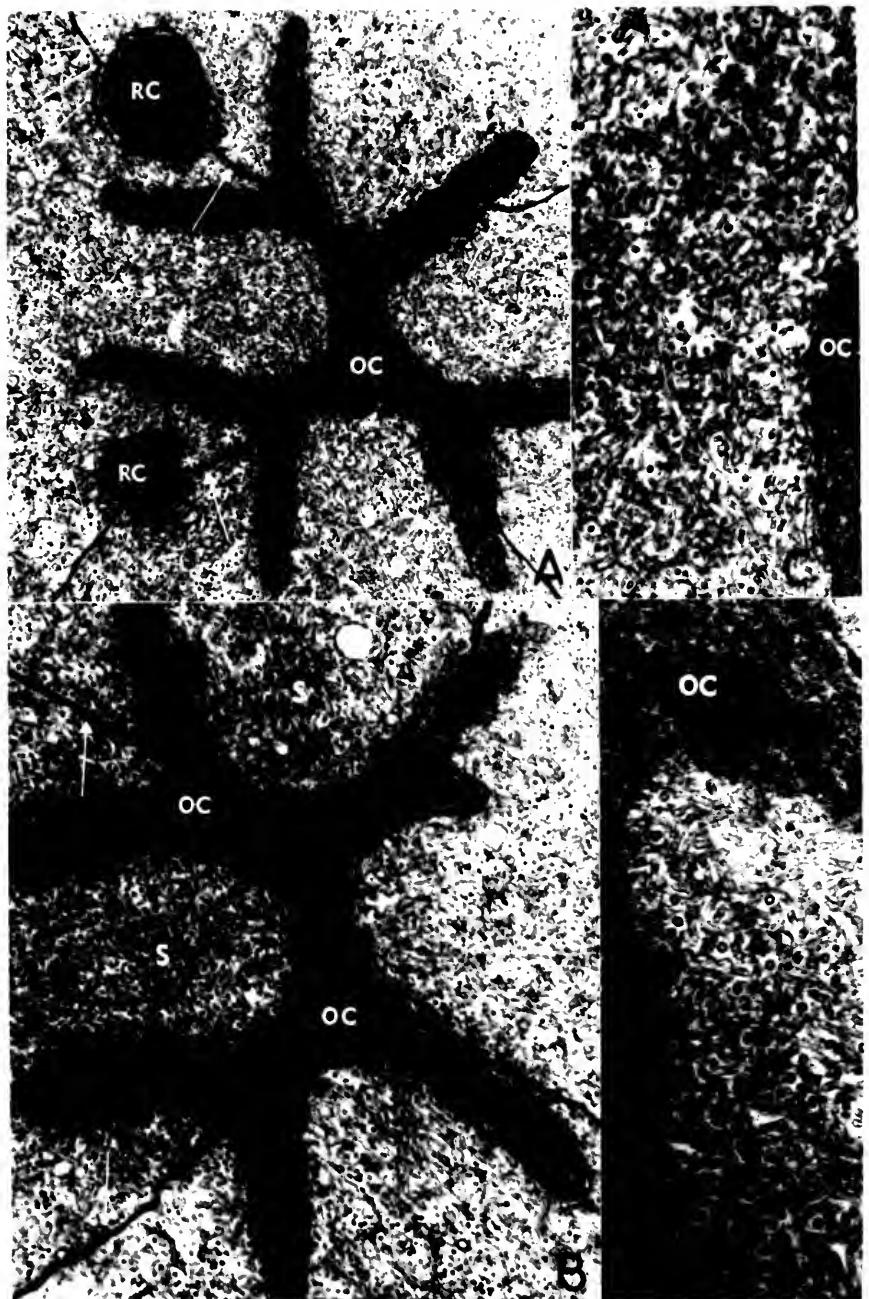


The proximal membrane of the Semper cell plate is invaginated; the ommatidial cavity (OC) and rhabdomere caps (RC) project into this invagination. Plate XII A is a cross section of the Semper cell plate showing 7 rhabdomere caps and the distal portion of the ommatidial cavity. This distal projection of the ommatidial cavity has 7 arms (Plate XIV A & B). Between these arms of the ommatidial cavity amorphous extracellular extensions of the rhabdomeres, the rhabdomere caps, are situated (Plate XII B). There are 7 rhabdomere caps, one corresponding to each rhabdomere. The trapezoidal configuration of the rhabdomere caps is the same configuration as the distal rhabdomeres. Plate XII is a cross section of the Semper cell plate. Four Semper cell nuclei (SN) and 7 rhabdomere caps (RC) are present in this section. The spot desmosomes that join the distal membranes of the Semper cells (see Plate XII A or XII B) are not present in this more proximal section. The spot desmosomes of the Semper cells and belt desmosomes (BD) that join the mesial face of the retinular cells (Plate XVII) differ primarily in length. The spot desmosomes of the Semper cells form localized plaques.

The tip of the extracellular rhabdomere caps (RC) project distally between the arms of the ommatidial cavity (OC) (Plate XIV A). The ommatidial cavity has 7 distal arms; between these arms the rhabdomere caps end. The

Plate XIV. Cochliomyia hominivorax unirradiated

- A. Cross section of the Semper cell junction and 7-armed ommatidial cavity (OC). The rhabdomere caps (RC) appear first between the arms of the ommatidial cavity. Note junction of Semper cell (arrows). X22800
- B. Cross section of the Semper cell (S) junction and 7 arms of the ommatidial cavity (OC). Note tubules in the Semper cell cytoplasm. X3800
- C. Cross section of Semper cell cytoplasm (S) junction near the ommatidial cavity (OC). Cytoplasm completely filled with microtubules. X51400
- D. Higher magnification cross section of Semper cell cytoplasm (S). Tubules completely fill the cytoplasm. X57000



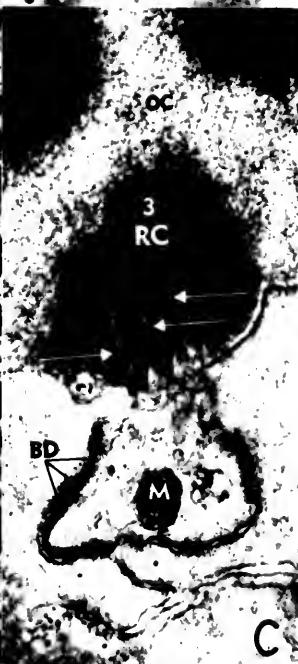
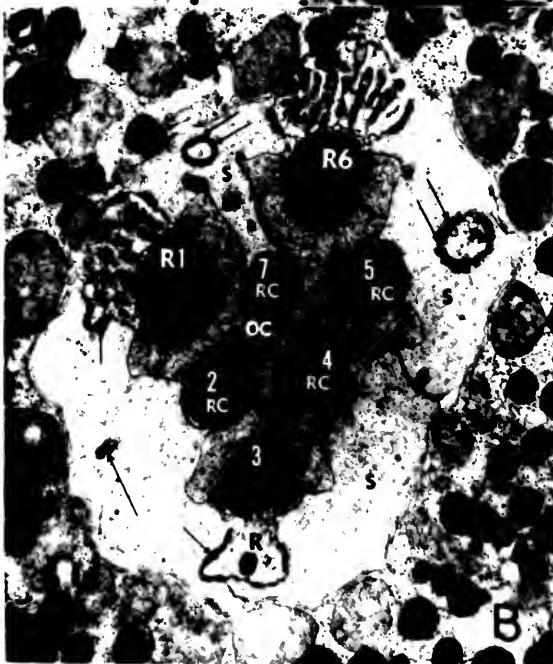
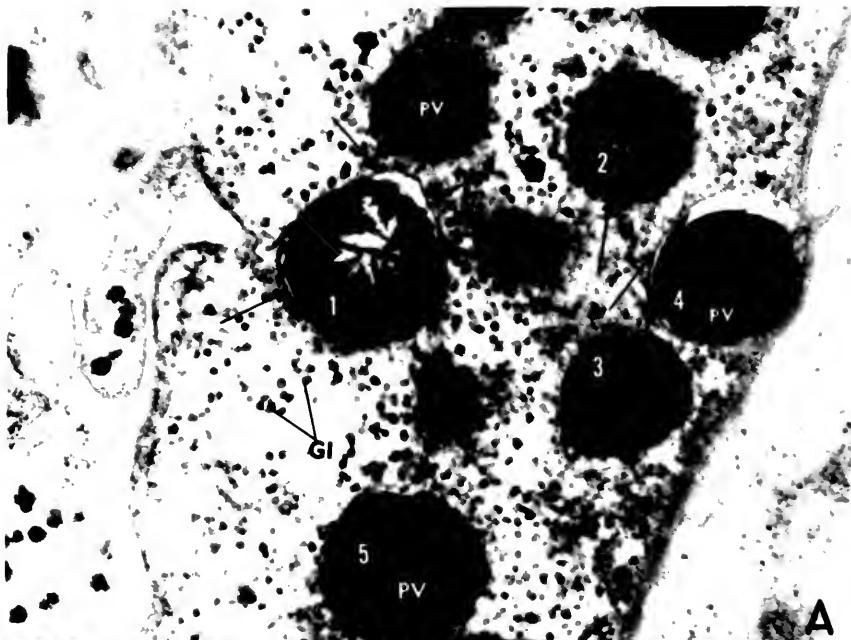
ommatidial cavity is located at the junction of the 4 Semper cells (S) (Plate XIV A, B). Plate XIV A and B are cross sections of the proximal portion of the junction of the Semper cell plate. The ommatidial cavity (OC) is formed by an invagination of the basal surface of the Semper cells. Electron dense granular material fills the ommatidial cavity (Plate XIV A, B, & D). The gap junctions (arrows, Plate XIV A, B) joining the Semper cells separate at the rhabdomere caps (RC) and reform on the other side of the cap (small arrows, Plate XIV A).

The cytoplasm of the Semper cells is totally devoid of organelles. Plate XIV has four different magnifications of Semper cell cytoplasm. Microtubules completely fill the Semper cells and no other organelles were observed. The microtubules are randomly packed and have no apparent orientation.

Plate XV B is an electron micrograph of a cross section at the junction of the Semper cells and the retinular cells. At this level the transition from rhabdomere cap (RC) to rhabdomeric microvilli occurs. Rhabdomere R3 is sectioned through the point of transition and shows both the amorphous cap (RC) and rhabdomeric microvilli (arrows, Plate XV C). The distal end of a retinular cell is attached to the proximal membrane of the Semper cell by a pointed evagination of the retinular cell membrane with desmosomal contact completely surrounding it (arrows, Plate XV B).

Plate XV. Cochliomyia hominivorax unirradiated

- A. Section through several pigment-filled vacuoles (PV) in a large pigment cell. Although generally referred to as pigment "granules" a vacular membrane (arrows) is present surrounding the pigment. Note growth of pigment crystals in vacuoles numbered 1-5. Note granular inclusions (GI) in cytoplasm found only in large pigment cells. X51300
- B. Cross section of a junction between Semper cells (S) and retinular cells (R). Four extracellular rhabdomere caps (RC), R2, R4, and R5, are present in the ommatidial cavity (OC). The rhabdomeres (R) of R1 and R6 are present and R3 is at the point of transition. Distal prolongations of retinular cells project into Semper cells and are joined by circular belt desmosome (arrows). X15200
- C. Higher magnification of retinular cell R3. Note rhabdomeric cap (RC), microvilli (arrows), mitochondria (M), and belt desmosome (BD). X38000



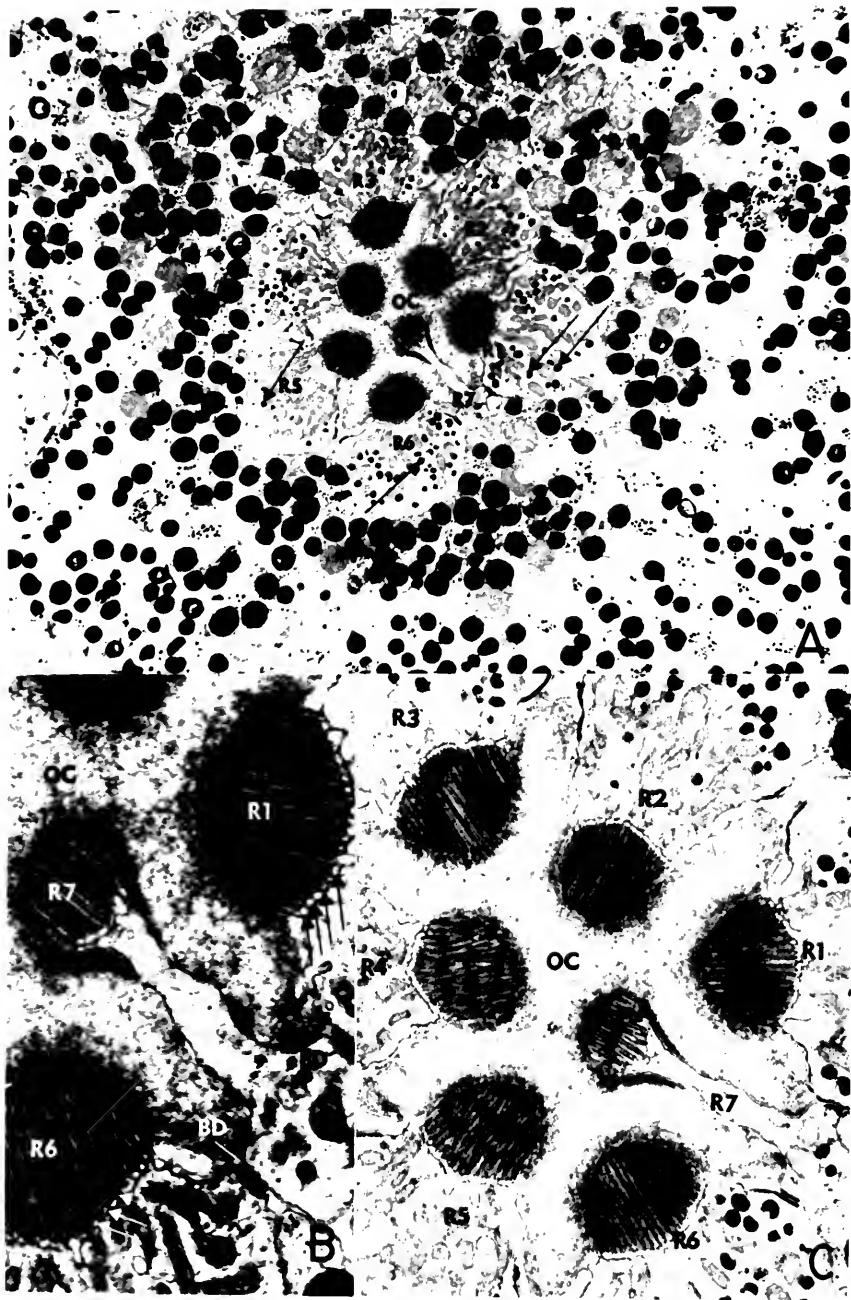
Pigment Cells

Two types of pigment cells are present surrounding the pseudocone and distal ommatidium (Fig. 4). These consist of 2 primary pigment cells (PP) and 6 large pigment cells (LPC) that extend from the middle of the pseudocone proximally to near the basement membrane. These 2 pigment cell types and their processes may be distinguished by dense granular inclusions (GI) that occur only in the cytoplasm of the large pigment cells (Plate XII, arrows; XV A). The nuclei of the large pigment cells (PN) are situated near the distal end of the ommatidium (Plate XIII B). There is a third type of pigment cell located at the basement membrane (Plate XIX A, Fig. 4). Four processes of these basal pigment cells (BPC) occlude the ommatidial cavity at the basement membrane (Plate XIX A).

Pigment-filled vacuoles are present in the cytoplasm of the primary pigment cells, large pigment cells, and basal pigment cells. The retinular cells also contain pigment-filled vacuoles (arrows, Plate XVI A). Plate XVI A shows both types of pigment vacuoles, the small retinular cell vacuoles (arrows), and the larger pigment cell vacuoles. Referred to as pigment "granules" by previous authors, they are actually vacuoles filled with pigment crystals. Plate XV A is an electronmicrograph of several pigment vacuoles (PV) in the cytoplasm of a large pigment cell (note the

Plate XVI. Cochliomyia hominivorax unirradiated

- A. Cross section of an ommatidium, primary pigment cells (PP), and large pigment cells (LP) just below the Semper cells. Note the presence of pigment filled vacuoles in the retinular cells (arrows). X9500
- B. Cross section of a portion of retinular cells R1, R6, and R7. The rhabdomeric microvilli are attached to the retinular cells by thin necks creating an extracellular space at their base (arrows). Note belt desmosomes (BD). X28500
- C. Cross section through distal ommatidium (higher magnification of plate XVI A). The retinular cells are joined by belt desmosomes the entire length of the ommatidial cavity (OC). Note microvillar orientation and rotational asymmetry of rhabdom. X22800



presence of granular inclusions that identify it as a large pigment cell). The vacuolar membrane (arrows) is clearly visible around pigment vacuoles that are incompletely filled with pigment crystals (Plate XV A). Apparently the pigment crystallizes within the vacuole and the vacuole fills with these pigment crystals. Long needle-like crystals are present in vacuoles 1 and 2. The vacuoles in Plate XV A numbered from 1-5 indicate different states of maturation. Vacuole 5 is considered to be a "mature" vacuole.

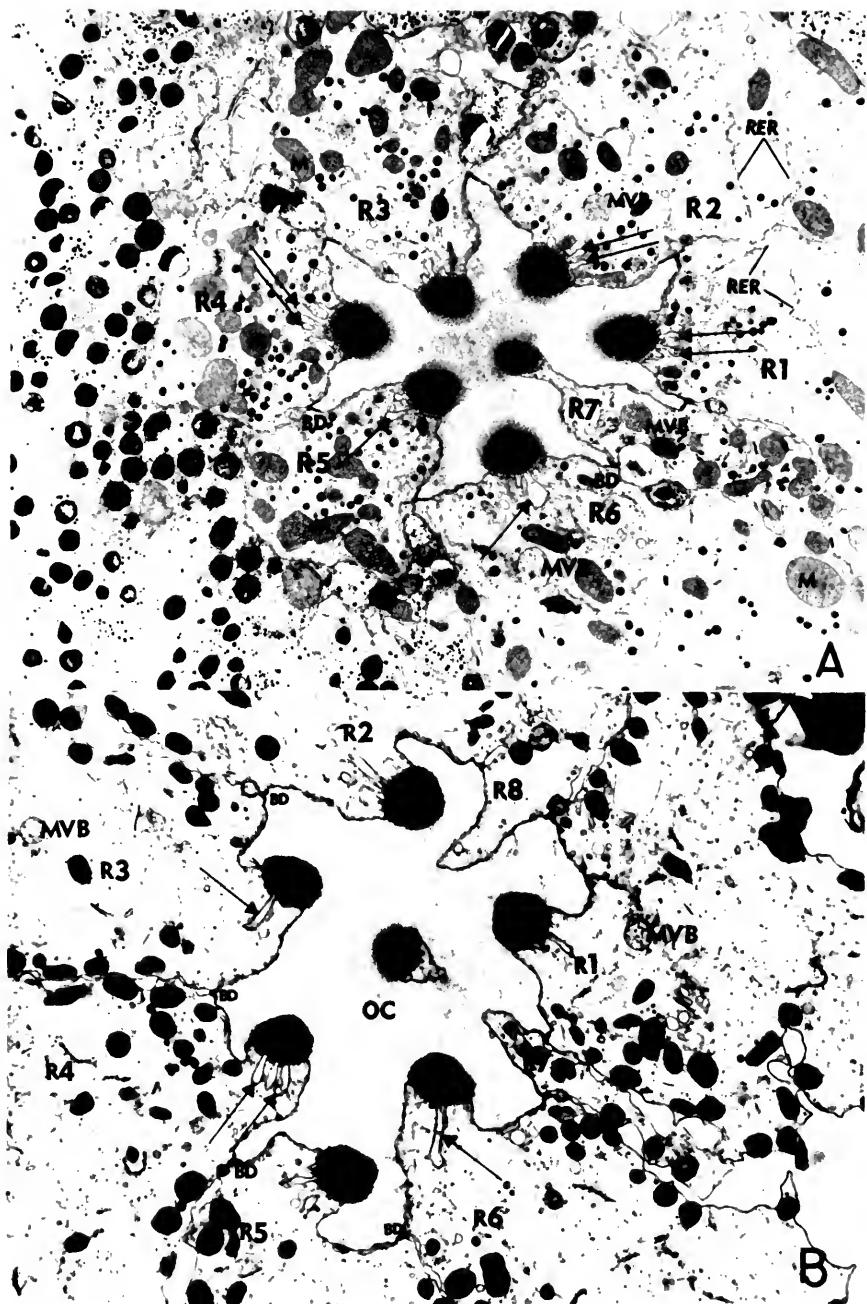
Retinular Cells

Each ommatidium has 8 retinular cells: 6 distributed peripherally around the ommatidial cavity (R1-R6) and 2 (R7 and R8) that project into the central ommatidial cavity. The retinular cells have two distinct regions: a soma or cell body that bears the microvilli, and an axonal segment that enters the first synaptic loci of the brain. The peripheral retina is composed of the retinular cell somata.

The retinular cells are joined by belt desmosomes (BD) that fuse the retinular cells for the entire length of the ommatidium (Plate XVI B, XVII A, B). The 7 belt desmosomes are the only points of attachment between the retinular cells. Pigment cell processes are present between the nondesmosomal membranes of the retinular cells.

Plate XVII. Cochliomyia hominivorax unirradiated

- A. Cross section of an ommatidium midway in the peripheral retina. The rhabdomere of the superior central cell, R7, is round and centrally located. The remaining rhabdomeres, R1-6, are conical. Invaginations of the plasma membrane beneath the microvilli form intracellular channels (arrows). Retinular cell cytoplasm contains pigment vacuoles, mitochondria (M), multivesicular bodies (MVB), and isolated cisternae of rough endoplasmic reticulum (RER). X8740
- B. Cross section of rhabdom at the junction of the superior central cell (R7) and the inferior central cell (R8). Note intracellular channels (arrows) and multivesicular bodies (MVB). X8740



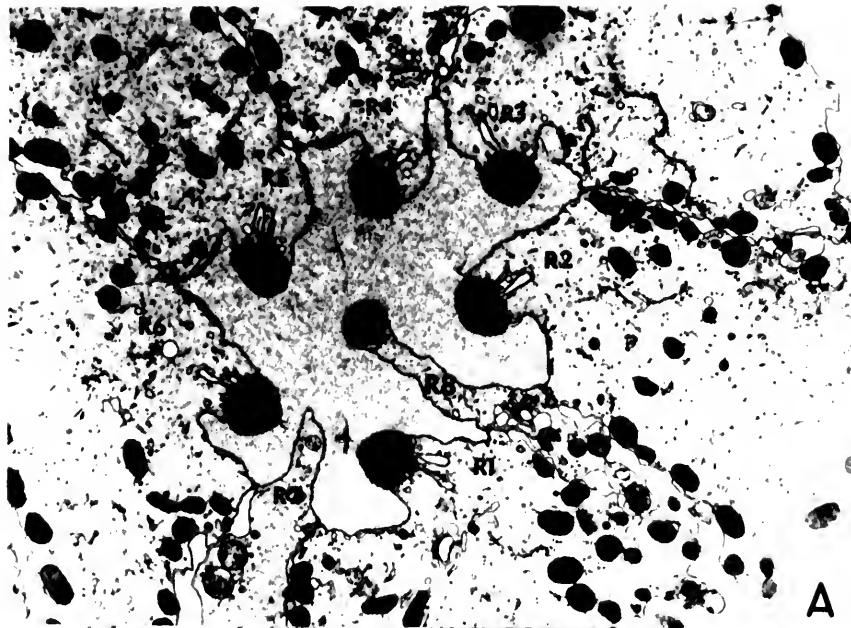
The arrangement of retinular cells R1-R6 is fixed in the distal portion of the rhabdom. The pattern is roughly trapezoidal and is rotationally asymmetrical. Plate XVI A and B are cross sections of an ommatidium passing directly beneath the Semper cells. The trapezoidal pattern disappears near the basement membrane (see Plate XVII B, XVIII A). This pattern is also present in the rhabdomere caps (Plate XIII B).

The configuration of this trapezoidal arrangement is such that a line inscribed through R3, R2, and R1 will be perpendicular to the horizontal plane of the eye and point toward the midline of the eye. This line (R3-R2-R1) is always parallel to the axis of the microvilli of the superior central cell (R7). The blind ends of the microvilli of R7 always point away from the midline of the eye. The superior cell microvilli in the dorsal portion of the eye point upwards, and those in the ventral hemisphere of the eye point down. This mirror image inversion has been found in other dipterans (Trujillo-Cenoz, 1972). An electron micrograph can be oriented using the microvilli of the superior central cell.

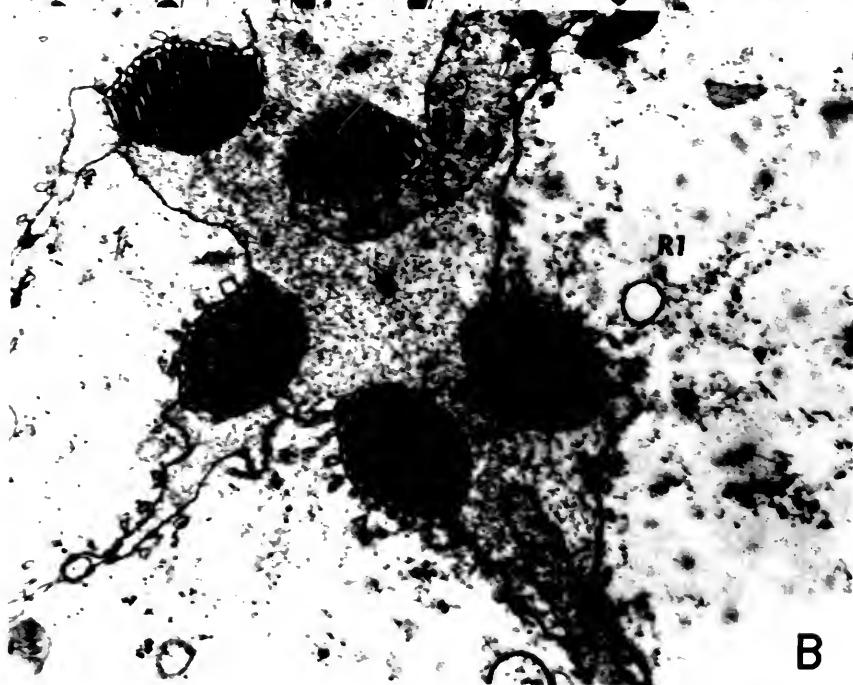
The ommatidial cavity (OC) (Plate XVI) extends the entire length of the rhabdom and is filled with an unknown material. This electron dense material completely fills the ommatidial cavity distally (Plate XV B; XIV A, B; XIII B). Directly beneath the Semper cells it forms clouds of

Plate XVIII. Cochliomyia hominivorax unirradiated

- A. Cross section of ommatidium just below the transition from superior central cell (R7) to inferior central cell (R8). Orientation of central cell microvilli is orthogonal. X8740
- B. Cross section of a portion of an ommatidium just below the transition from the superior central cell (R7) to the inferior central cell (R8). Microvillar orientation of central cell rhabdomeres is parallel. X22800



A



B

electron dense material around the rhabdomeres (Plate XVI B, C). Near the midline of the eye this material persists around the rhabdomeres (Plate XVII A), but near the transition zone from the superior central cell (R7) to the inferior central cell (R8) it is evenly dispersed in the ommatidial cavity and shows no affinity for the rhabdomeres (Plate XVII B; XVIII A). The ommatidial cavity is sealed at the basement membrane by processes from the 4 basal pigment cells (Plate XIX A), laterally by the belt desmosomes fusing the retinular cells and distally by the occluded gap junction and spot desmosomes of the Semper cell membranes of the Semper cell plate.

The rhabdomeric microvilli are borne on thin neck-like stalks (Plate XVI B). The central cells bear between 13-15 longitudinal rows of microvilli and the peripheral rhabdomeres (R1-R6) have between 22-24 rows of microvilli (Plate XVI). The rhabdomeric microvilli are oriented perpendicularly to the longitudinal axis of the rhabdom and therefore perpendicular to the light path. The long axes of the microvilli of R1 and R4 are parallel, as are the axes of R2 and R5 and R3 and R6 (Plate XVI C and XVII A).

The rhabdomeres of retinular cells R1-R6 have the shape of a truncated cone in cross section (Plate XVI A, C; XVII A, B; XVIII). The peripheral rhabdomeres (R1-R6) retain this shape throughout their entire length. The central

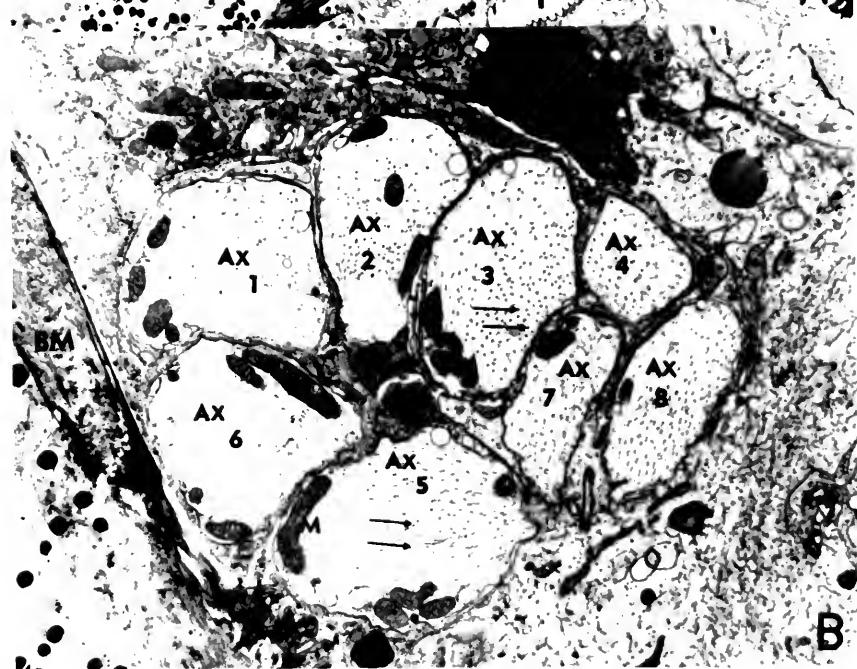
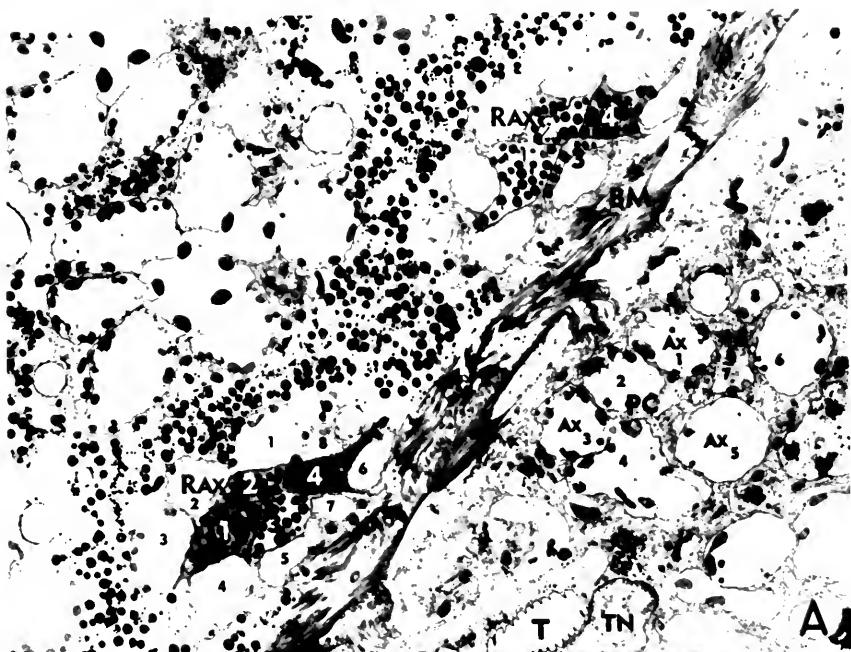
rhabdomere is round in cross section (Plate XVI A, B, C; XVII). The seventh centrally located rhabdomere actually consists of the rhabdomeres of the 2 retinular cells R7 and R8 (Plate XVII, B, XVIII). The distal cell (R7), the superior central cell (SCC), bears a rhabdomere approximately twice as long as the inferior central cell (R8, ICC). The rhabdomere of the inferior central cell (R8) is directly below the rhabdomere of the superior central cell (R7) forming a single central rhabdomere (Plate XVII B). The inferior central cell is located between the peripheral retinular cells R1 and R2 (Plate XVII B).

The rhabdomeres can be divided into two populations by the orientation of the central rhabdomere microvilli. In one group the microvilli of the superior and inferior central cells are orthogonal (Plate XVII B) and in the other group the microvilli are parallel (Plate XVIII A).

The retinular cells contain small pigment vacuoles (arrows, Plate XVI A) similar in structure to those of the primary, large, and basal pigment cells. Retinular cell pigment vacuoles are restricted to the distal portion of the superior central cell and are not present near or below the transition zone from the superior central cell to the inferior central cell (compare A & B of Plate XVII). Numerous mitochondria (M) are present in the cytoplasm of the retinular cells (Plate XVII A). Sparse and isolated cisternae of

Plate XIX. Cochliomyia hominivorax unirradiated

- A. Oblique section through the basement membrane (BM). Near the basement membrane the ommatidial cavity (O) is filled by 4 processes of the basal pigment cells (1-4). Below the basement membrane are bundles of axons, the pseudocapsules (PC), trachea (T), tracheoblast nuclei (TN). X3800
- B. Cross section of a single pseudocartridge directly beneath the basement membrane (BM). The retinular cell axons (Ax 1-8) contain mitochondria (M) and numerous microtubules (arrows). X7600



rough endoplasmic reticulum (RER) are present in the cytoplasm but few ribosomes are attached to them. Multivesicular bodies (MVB) are often encountered in the cytoplasm of the retinular cells (Plate XVII A, B). Deep invaginations of the plasma membrane (arrows) form long membranous channels and pinocytotic vesicles at the base of the rhabdomeric microvilli (Plate XVII A, B; XVIII A).

Lamina

Near the basement membrane (BM) the retinular cells lose their rhabdomeres and pass through the basement membrane into the lamina ganglionaris. Plate XIX A is an electron micrograph of a section through the area of the basement membrane. The ommatidial cavity is filled by four processes (1-4) of the basal pigment cells or sacs. The external layer of the lamina, the fenestrated layer, contains both numerous tracheae (T), and bundles of retinula cell axons, the pseudocartridges (PC). Eight axonal fibers (Ax 1-8) derived from the retinular cells form the pseudocartridges (Plate XIX B) and contain numerous mitochondria (M) and longitudinally oriented microtubules (arrows) (Plate XIX B).

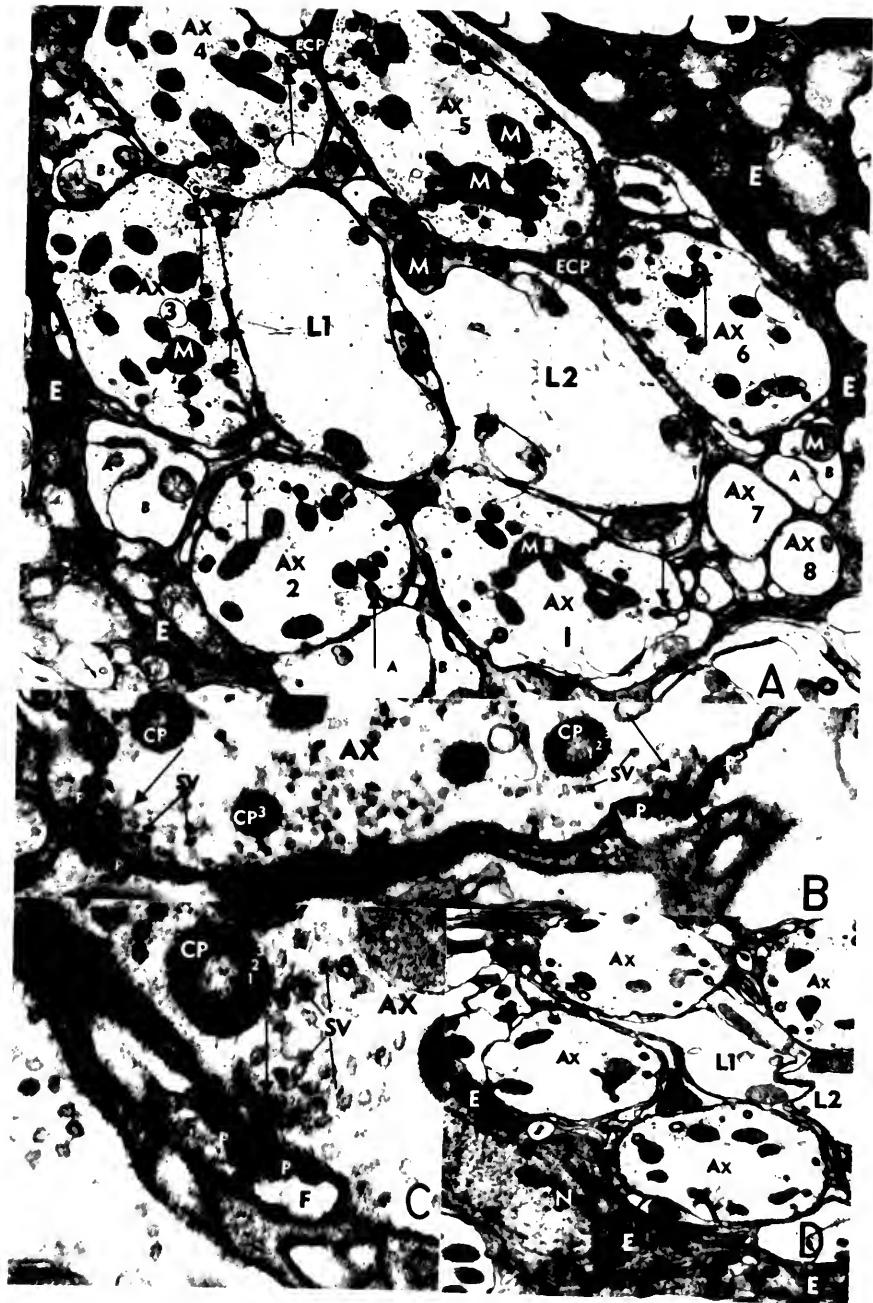
The axons derived from the 6 peripheral retinular cells (R1-R6) separate below the pseudocartridges and enter the external plexiform layer of the lamina. The external plexiform layer consists of units containing 2 second order neurons surrounded by 6 photoreceptor axons, termed optic

cartridges. Plate XX A is a cross section of an optic cartridge showing the 2 second order neurons (L1 and L2) and 6 retinula cell axons (Ax 1-6) surrounding them. The axons from the superior central cell and the inferior central cell (Ax 7 and Ax 8) are displaced laterally bypassing the lamina and synapse directly with the medulla. Each photoreceptor axon (Ax 1-6) is associated with a pair of axons (A & B) termed the centrifugal fibers (Trujillo-Cenoz, 1965b). The optical cartridges are separated by epithelial glial cells that surround the cartridges (Plate XX A & D). Processes from the glial cells penetrate between the peripheral retinula cell axons, Ax 1-6. In addition, there are specialized glial projections, the capitate projections (CP), which project into Ax 1-6. The capitate projections are formed by the evaginations of the glial cell process membrane into axons 1-6 (Plate XX A, B, & C). The capitate projections have a narrow "stalk" and up to 3 terminal masses. Plate XX B and C show sections of terminal masses (CP) composed of (1) an inner membrane or glial cell process membrane, (2) an outer membrane or the invaginated axonal membrane, and (3) an electron dense covering. A "grazing" section of the electron dense coat of a terminal mass (CP3) is shown in Plate XX B.

The T-shaped synaptic ribbons identified by previous authors as chemical synapses (Trujillo-Cenoz, 1965a, 1965b; Boschek, 1971) are present in the retinula cell axons.

Plate XX. Cochliomyia hominivorax unirradiated

- A. Cross section of an optical cartridge. The cartridge consists of 2 second order axons (L1 and L2) surrounded by 6 retinular cell axons (Ax1-6). Paired axons of the central cells (Ax7 and Ax8) are displaced laterally as are the centrifugal cell axons (A and B). Numerous mitochondria (M) and specialized glial processes, the capitate projections (arrows), derived from the epithelial glial cells (E) are prevalent in Ax1-6. X15200
- B. Synaptic loci or T-shaped synaptic ribbons (arrows). Presynaptic axon (ax) is filled with synaptic vesicles (SV). Note trilaminate head piece of capitate projection (1, 2, 3). X50000
- C. Single synaptic ribbon. Presynaptic vesicles (SV) and T-shaped synaptic ribbon (arrow) are in the presynaptic axon (Ax). Post synaptic fibers (F) contain dense plate-like structures (P). Note capitate projections (CP) through layered terminal head (1, 2, 3). X93100
- D. Cross section of an optical cartridge adjacent epithelial glial cell (E) and glial nucleus (N). X22800



They consist of an electron dense T-shaped synaptic ribbon and synaptic vesicles contained in the presynaptic fiber, a retinula cell axon. In Plate XX B and C the retinula cell axons contain numerous synaptic vesicles and T-shaped synaptic ribbons (arrows). The post-synaptic axonal fibers (F), usually L1 or L2 or processes of L1 or L2, contain hollow plate-like structures surrounded by electron dense material.

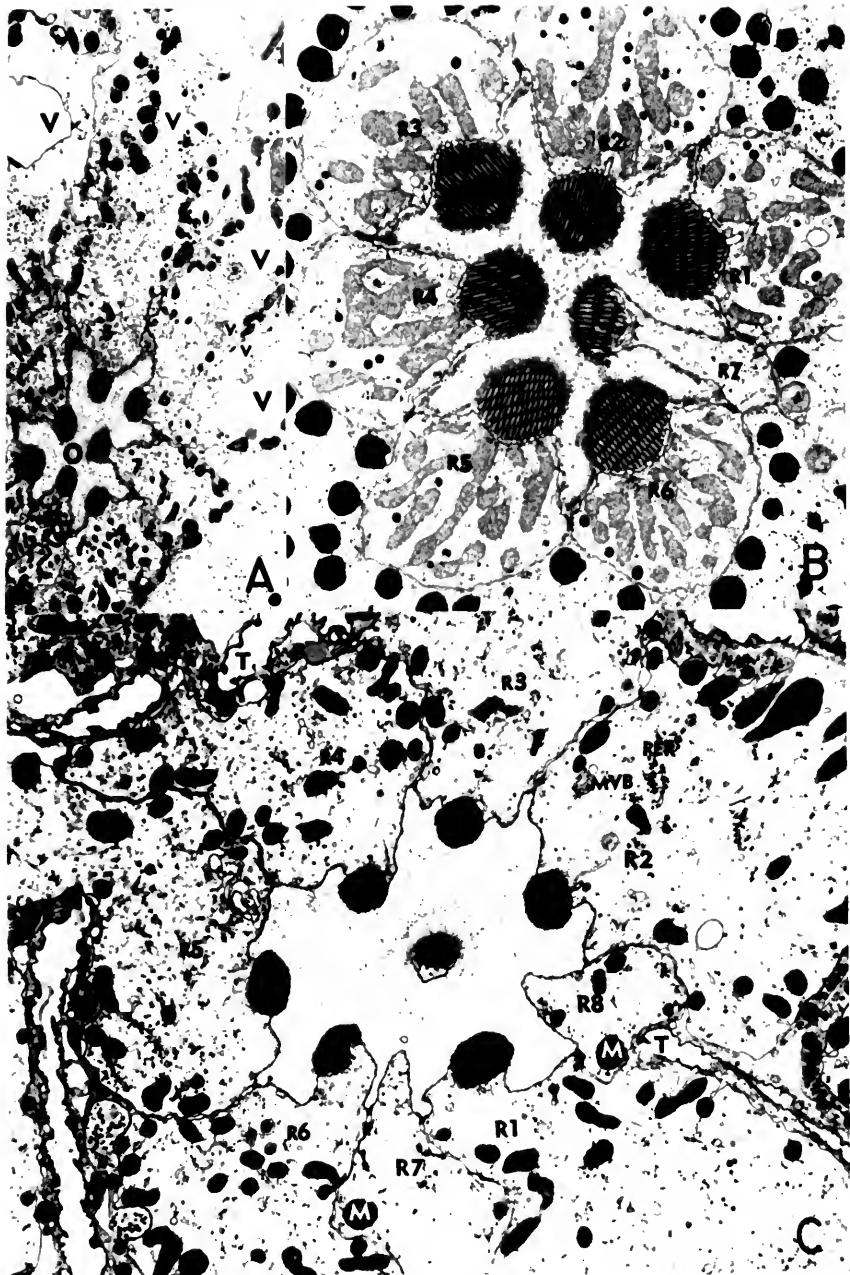
Irradiated Flies

The screwworm flies that were mass reared on an artificial diet, sterilized, and shipped to Gainesville from Mission, Texas, showed a number of abnormalities. Three types of abnormalities were encountered: (1) highly vacuolate "thin" retinular cell cytoplasm, (2) abnormally shaped rhabdomeres, and (3) large vacuoles in the axoplasm of the retinular cell axons in the external plexiform layer. One striking aspect of the mass-reared flies was the extreme variability between weekly shipments and within a shipment. Many of the eyes examined appeared normal while others within the same group were abnormal.

Plate XXI A is a cross section of an ommatidium from an irradiated fly. The rhabdomeres appear normal in both shape and relative position; however, the cytoplasm of the retinular cells is very thin, highly vacuolated, and devoid

Plate XXI. Cochlimyia hominivorax irradiated

- A. Oblique section through the distal portion of an ommatidium. The cytoplasm of the retinular cells contains large atypical vacuoles (V). Note mirror image reversal in numbering. X3800
- B. Cross section through the distal portion of an apparently normal irradiated ommatidium. X11970
- C. Cross section of an apparently normal R7-R8 central cell transition of an irradiated ommatidium. Note mitochondria (M), multivesicular bodies (MVB), and isolated cisternae of rough endoplasmic reticulum (RER). Trachea (T) numerous and impinge on the retinular cells. X8740.

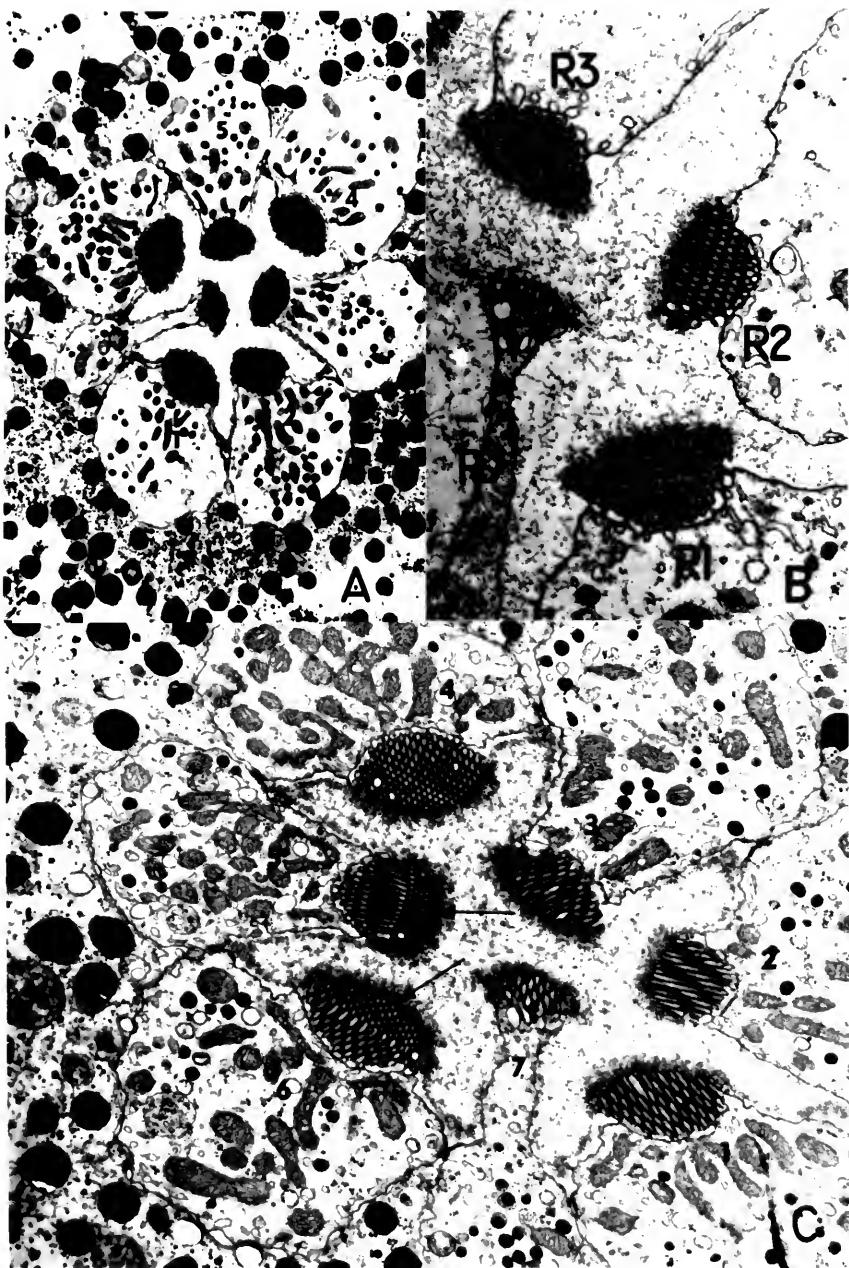


of small retinular cell pigment vacuoles. Plate XX B is a cross section of the distal end of an ommatidium just below the Semper cells. These irradiated rhabdomeres appear normal. Plate XXI C is an electron micrograph of an apparently normal central cell transition zone (R7-R8). The cytoplasm is thin and several tracheae (T) impinge on the ommatidium (compare with normal ommatidia in Plate XVII and XVIII and note the absence of trachea). Intracellular channels and pinocytotic vesicles generally present at the base of the microvilli are considerably reduced when compared to the unirradiated flies.

Plate XXII illustrates the most common abnormality encountered in the irradiated flies examined. The rhabdomeres of these abnormal ommatidia are obconical in shape (compare with the normal truncated cone shape of normal rhabdomeres Plate XVI and XVII). The abnormal obconical rhabdomeres have fewer rows of microvilli than the normal unirradiated rhabdomeres. The peripheral rhabdomeres (R1-R6) have between 15-17 longitudinal rows of microvilli compared with a normal complement of 22-24 and the central rhabdomere has between 10-11 longitudinal rows of microvilli rather than the normal 13-15 rows. The rhabdomeres of the retinular cells shown in Plate XXII C are partially obconical and the rhabdomeric microvilli of R5 and R6 (arrows) are oriented in two planes. The microvilli appearing in the cross section appear to be bent since they are

Plate XXII. Cochliomyia hominivorax irradiated

- A. Cross section of an aberrant irradiated ommatidium.
Note abnormal obconical shape of the rhabdomeres.
X8740
- B. Cross section of a portion (R7, 1, 2, and 3) of
an abnormal irradiated ommatidium. Rhabdomeres
are obconical in shape and microvilli are greatly
reduced in number. X22800
- C. Cross section of an abnormal irradiated ommatidium.
Rhabdomeres and microvilli are abnormally shaped.
The microvilli of R5 and R6 (arrows) are bent.
Note abnormal number of trachea present. X17100



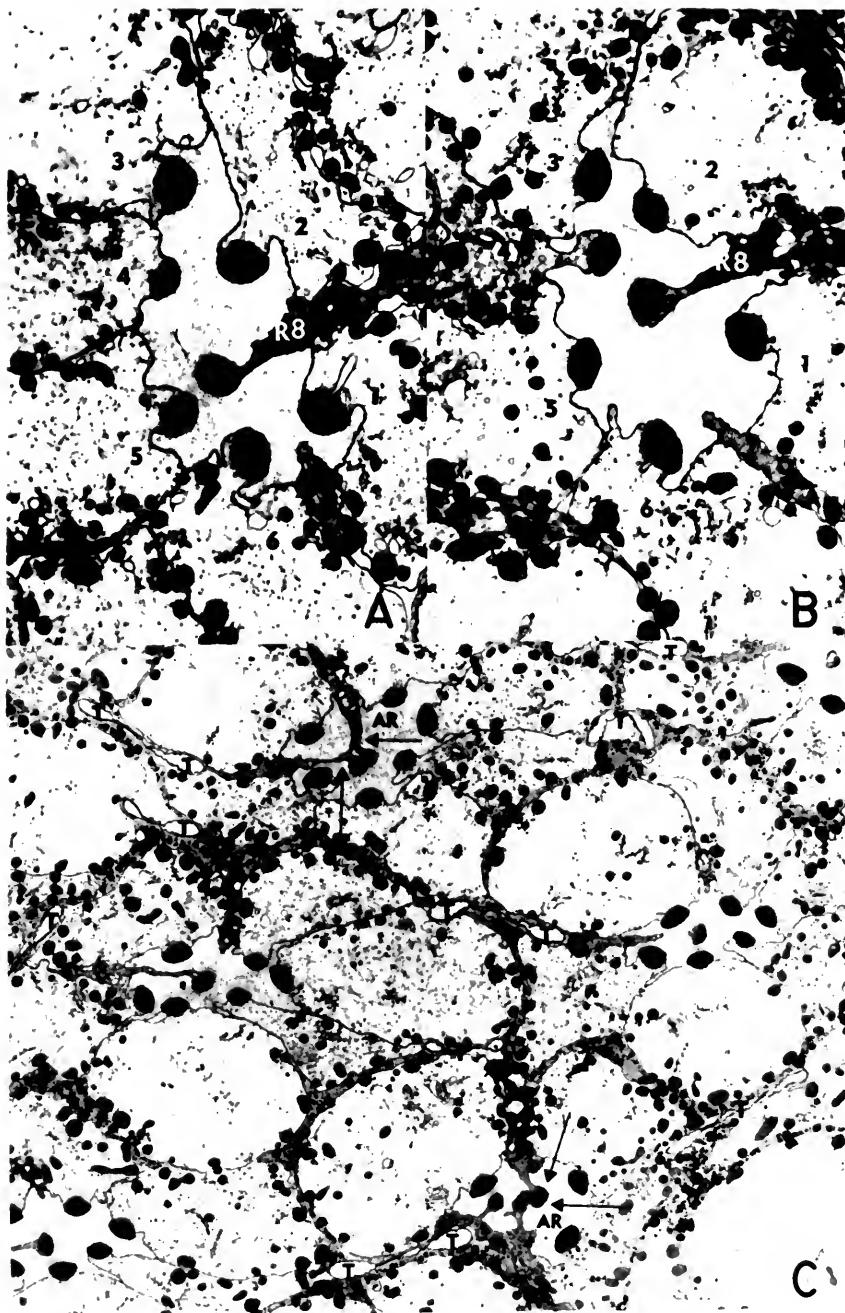
observed both longitudinally and transversely. Normal microvilli are straight and their longitudinal axis should always be perpendicular to the light path.

Plate XXIII A and B are cross sections of two normal appearing ommatidia from an irradiated fly below the transition zone. These photomicrographs have been included since they show the relative orientation of the rhabdomeric microvilli of the superior and inferior central cells (R7 and R8). The central cell rhabdomere microvilli in XXIII A are parallel and the rhabdomeric microvilli in XXIII B are orthogonal.

In Plate XXIII C two abnormal transition rhabdoms (AR) are indicated by arrows. This photomicrograph is of a cross section through the transition zone and shows an abundance of tracheae not present in the unirradiated flies. Plate XIV A and B are higher magnification photomicrographs of the two abnormal rhabdoms of Plate XXIII C. The rhabdomeres are obconical as well as showing abnormal R7-R8 central cell transition zones. Plate XXIII A shows a rhabdom with only 6 rhabdomeres, 2 of these are touching in the central part of the ommatidial cavity. The touching rhabdomeres should be numbers R7 and R8, the central cells; however, since there are 2 rhabdomeres between the 2 central cells they cannot be R7 and R8. The abnormal rhabdom shown in Plate XXIV C has 8 rhabdomeres present, 1 more

Plate XXIII. Cochliomyia hominivorax irradiated

- A. Cross section of 8 cell ommatidium. Microvilli of the superior and inferior central cells are parallel. X8740
- B. Cross section of 8 cell ommatidium. Microvilli of the superior and inferior central cells are orthogonal. X8740
- C. Cross section of several ommatidia through the transition zone of superior and inferior central cells. Note 2 abnormal (AR) transition ommatidia (arrows). X3800



than normal. The central cells lie side by side in the ommatidial cavity rather than the normal arrangement with the central cell rhabdomeres on top of one another. A and B of Plate XXIV show the presence of abnormal numbers of trachea (T) penetrating the space between the retinula cells and impinging on the rhabdom.

One of the abnormalities encountered in the irradiated flies were large vacuoles (V) in the axoplasm of the retinula cell axons at the level of the capsules in the external plexiform layer of the lamina (Plate XXV A and B). The two central second order axons appear to be normal. Plate XXV B is a higher magnification electron micrograph of 2 abnormal axons containing large vacuoles. The other components of the lamina appear to be normal.

Discussion

The microstructure of the peripheral retina and lamina of unirradiated Cochliomyia hominovorax is very similar to that of other genera of cyclorrhaphan Diptera previously studied. It would be very difficult to distinguish electron micrographs of Cochliomyia hominovorax from those published of the eyes of Musca (Boschek, 1971). Drosophila (Fuge, 1967), Chrysomia, Lucilia, and Sarcophaga (Melamed & Trujillo-Cenoz, 1968; Trujillo-Cenoz, 1965a, 1965b, 1969; Trujillo-Cenoz & Melamed, 1962, 1963, 1966a). The eyes of irradiated screwworm flies reared in

Plate XXIV. Cochliomyia hominivorax irradiated

- A. Cross section of an ommatidium with an abnormal superior-inferior central cell transition. Only 6 abnormal rhabdomeres are present and 2 of these, apparently R7 and R8, are touching. Note the abnormal number of trachea (T) impinging on the rhabdom. X8740
- B. Cross section of an ommatidium with an abnormal superior-inferior central cell transition. Eight rhabdomeres are present, 1 more than normal. Rhabdomeres R7 and R8 are joined and form a bridge. Note the abnormal number of trachea present. X8740

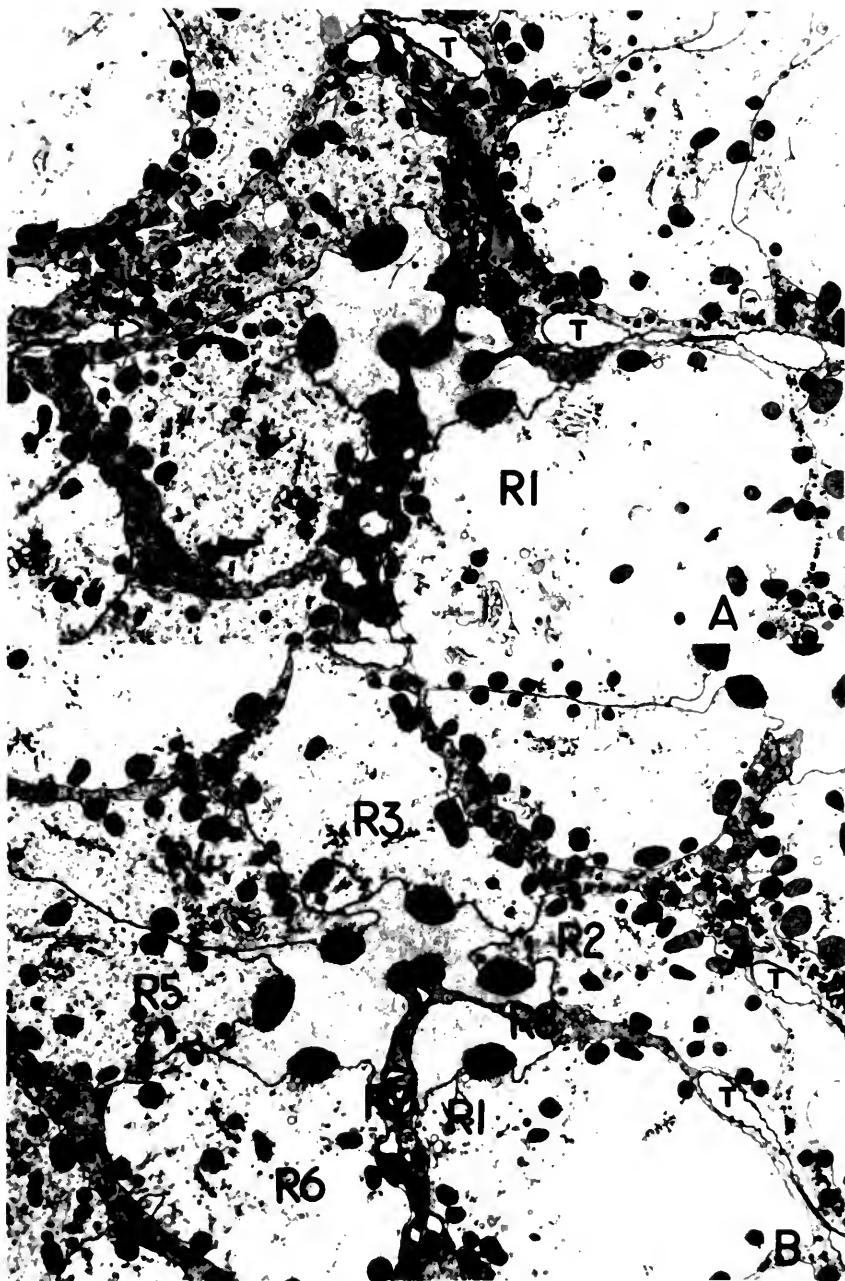
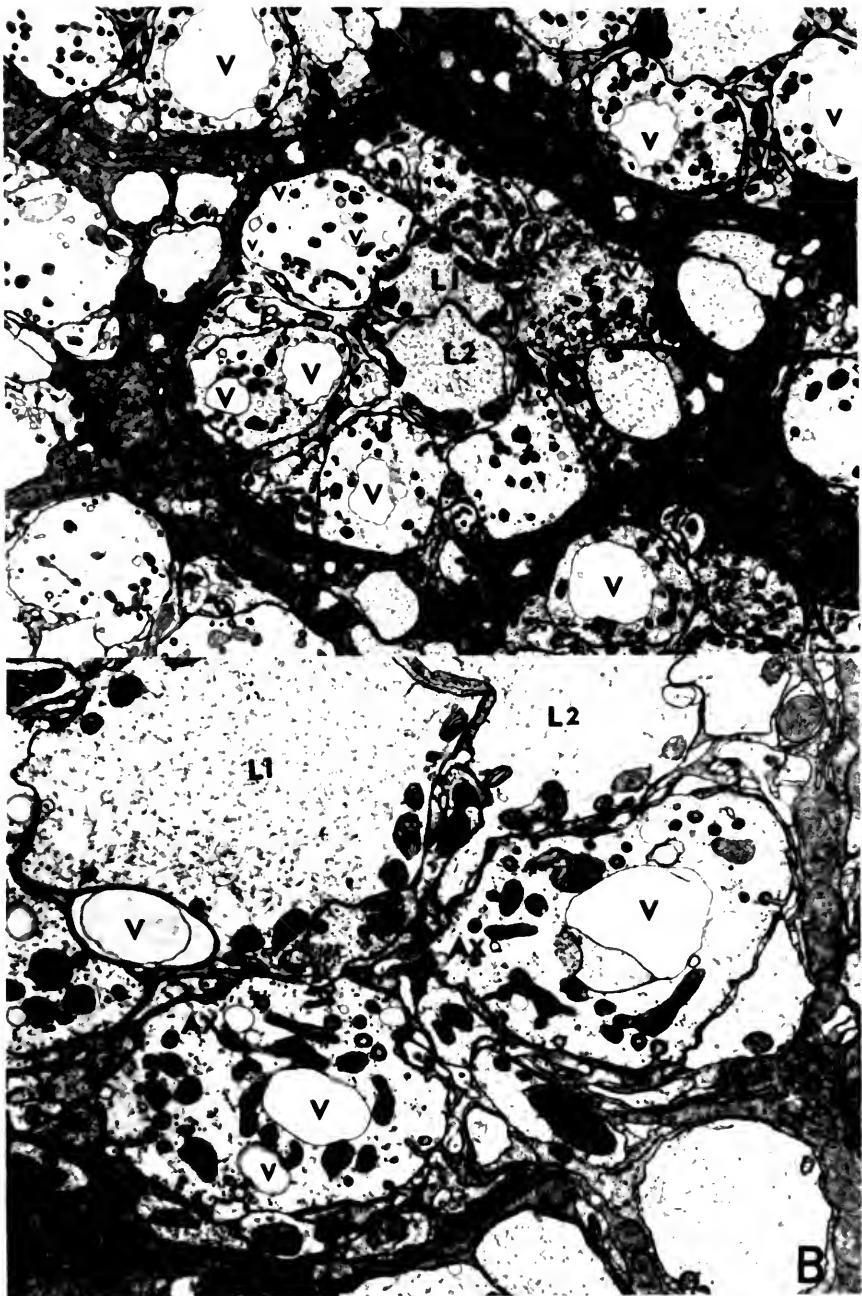


Plate XXV. Cochliomyia hominivorax irradiated

A. Cross section of an optical cartridge. The optical cartridges of irradiated flies contain large vacuolar spaces in the axoplasm (V). Second order axons, L1 and L2, appear normal. X5890

B. Higher magnification cross section of 2 photoreceptor axons (Ax) in the optical cartridge showing large abnormal vacuoles (V) in the axoplasm.
X11970



Mission, Texas, for mass release differed markedly in a number of respects from the unirradiated flies and the other dipteran previously studied genera.

Peripheral retina

The peripheral retina of Cochliomyia hominivorax is composed of ommatidia characteristic of the cyclorrhaphan diptera. Each ommatidium has the characteristic dioptric apparatus and open rhabdom. The organization of the 8 retinula cells, 6 peripheral and 2 central, is very characteristic of the other genera studied. The open rhabdom of the dipterans has been studied in greater detail than any other compound eye because Musca and Calliphora are used in many electrophysiological studies (Wehner, 1972).

This research has verified important prior studies and will contribute new observations.

Dioptric apparatus

The corneal lens of the eye of Cochliomyia is very similar to that of Sympycnus lineatus (Trujillo-Cenoz, 1972) and Calliphora (Seitz, 1968). The small rounded elevations that cover the surface of the lens are characteristic of dipterans previously studied but are not considered the equivalent of corneal nipples found in Depidoptera (Bernard & Miller, 1968; Bernhardt *et al.*, 1972). The lens has alternating dense and rare layers (Plate X) that have been described as quarter wavelength interference

filters in the horsefly (Bernard & Miller, 1968) and are common in many dipterans (Trujillo-Cenoz, 1972).

In Cochliomyia and other diptera with pseudocone eyes, the cone is a gelatinous material described as being isotropic by previous authors (Seitz, 1968; Trujillo-Cenoz, 1972). The pseudocone of Cochliomyia is not entirely isotrophic and has a diffuse area of greater electron density in the center of the pseudocone. This electron dense material may be analogous to the electron dense core at the junction of the 4 cone cells in the eucone eyes of insects (Fischer & Horstmann, 1971).

The Semper Cells

The function and origin of the Semper cells is not fully understood. The most obvious function is the closure of the bottom of the pseudocone cavity. The 4 Semper cells are wedge-shaped and form a transparent flattened plate (XIII B) that occludes the proximal end of the pseudocone cavity. The other postulated function is to "couple" the dioptric apparatus to the retinula cells (Boschek, 1971; Trujillo-Cenoz, 1972) and maintain proper optical spacing of the distal rhabdomeres (Boschek, 1971). The cytoplasm of the Semper cells of Cochliomyia and Musca (Boschek, 1971) is devoid of organelles. This lack of organelles is interpreted by Boschek (1971) as meaning the Semper cells are metabolically inactive. This is also the

case in Cochliomyia; no mitochondria, endoplasmic reticulum, golgi, or other organelles indicative of metabolic activity are present. Microtubules completely fill the Semper cells. In Musca the microtubules are oriented at an angle of 30° off the longitudinal axis of the ommatidium (Boschek, 1971). The microtubules in Cochliomyia show no orientation and are randomly packed. The function of these microtubules is probably cytoskeletal. They provide rigidity and resistance to deformation of the Semper cell plate. This rigidity is important in maintaining the trapezoidal conformation and dimensions of the distal end of the open rhabdom of C. hominovorax. Insecta with a closed rhabdom do not encounter the conformational maintenance problems of insects with open rhabdoms.

The trapezoidal rhabdomeric configuration is maintained throughout the visual system (Braitenberg, 1972; Trujillo-Cenoz, 1969). Mirror image symmetry of the trapezoidal pattern is constant in the 4 quadrants of the eye (Braitenberg, 1972) and indicates the importance of maintenance of the trapezoidal configuration in the distal rhabdom. In an open rhabdom each rhabdomere functions as an independent wave guide. Varela (1971) postulates the open rhabdom of dipterans resembles the vertebrate eye in this regard and is the most advanced compound eye in the arthropods. The open rhabdom is probably an excellent analyzer of polarized light (Varela, 1971) and the

trapezoidal rhabdromeric configuration probably represents an important part in the processing and analysis of visual stimuli.

The trapezoidal pattern is only maintained in the distal portion of the ommatidia and disappears near the basement membrane indicating that a distal structure, such as the Semper cells, is responsible for the conformation. The projection of the retinula cell axons over the lamina reproduces the trapezoidal pattern of the distal rhabdom in such a way that the axons from retinula cells whose microvilli are in the same plane end in the same optical cartridge (Trujillo-Cenoz, 1965b, 1972; Trujillo-Cenoz & Bernard, 1972; Kirschfeld, 1967). Several other structures appear to be very important in the maintenance of the rhabdromeric trapezoidal configuration. The distal ends of the retinula cells are firmly attached to proximal invaginations in the Semper cells (Plate XV B arrows). Pointed processes of the distal end of the retinula cells are anchored by desmosomes (Plate XV B arrows). The distal end of each rhabdomere is covered by an electron dense rhabdomere cap that extends distally from the rhabdomere along the side of the ommatidial cavity and into the rhabdromeric cap invaginations of the Semper cells between the cell junctions. These electron dense rhabdomere caps retain the trapezoidal pattern of the rhabdomeres (Plate XIII B) and probably assist in its maintenance.

This optically important configuration of the ommatidium is probably in great measure also conserved by the desmosomal and gap junctions joining the retinular cells and Semper cells. Desmosomes are generally present in cells where structural stability of form or attachment is important (Satir & Gilula, 1973). The retinula cells are joined their entire length by belt desmosomes and the Semper cells by spot desmosomes and gap junctions. These desmosomal connectives, Semper cell to Semper cell, retinula cell to retinula cell, and retinula cell to Semper cell help maintain the rigidity of the ommatidium plate.

The ommatidial cavity is filled with fluid, probably haemolymph or a derivative of the haemolymph. The subrhabdomeric intracellular channels and vacuoles may secrete fluid into the ommatidial cavity. This fluid may help hydrostatically maintain the ommatidial cavity since the eyes of dehydrated flies collapse. An extracellular electron dense amorphous substance is present in the rhabdomic fluid. The material completely fills the ommatidial cavity at its distal end where the ommatidial cavity occupies the invaginated proximal membrane of the Semper cell plate. Beneath the Semper cells the electron dense material is associated with the rhabdomeres forming a halo around the tips of the microvilli (Plate XVI B, C). The halo or cloud of electron dense material is not associated with the rhabdomeric microvilli below the midpoint of the ommatidium.

The distal membrane surface of the Semper cell plate is produced into a network of membranous projections or ridges (Plate XII). These ridges have been described by previous authors (Boschek, 1971; Trujillo-Cenoz, 1972) as short irregular microvilli. The function of these ridges is unknown but they may keep the gelatinous pseudo-cone from slipping or being displaced laterally. The ommatidial cavity occupies a 7-armed membranous invagination of the Semper cell plate's proximal membrane. Trujillo-Cenoz (1965a) and Boschek (1971) published electron micrographs of the distal end of the ommatidial cavity but failed to recognize it as such or mention it in the text of their articles.

Pigment Cells

Pigment filled vacuoles are present in the pigment cells and retinula cells of C. hominovorax. Pigment-filled vacuoles are referred to by prior authors as pigment "granules" (Trujillo-Cenoz, 1972; Boschek, 1971; Butler et al., 1970). In C. hominovorax a distinct vacuolar membrane surrounds the pigment (Plate XV A arrows). This vacuolar membrane has been previously reported in the eye of the toad bug, Gelastocoris occulatus by Burton & Stockhammer (1969). The pigment vacuoles of Gelastocoris contain needle-like pigment crystals similar in appearance to those in the pigment vacuoles of the screwworm fly. A process of

pigment crystal precipitation within the vacuoles and subsequent filling or maturation of the vacuole appears to occur. Plate XV A shows 5 pigment vacuoles, numbered 1-5, in various states of maturation.

The origin of the basal pigment cells is unknown. Processes of the basal pigment "cells" fill the bottom of the ommatidial cavity (Plate XIX A) and are present between the basal ommatidia at the basement membrane.

Brammer (1970) in Aedes aegypti described processes from the 4 Semper cells as terminating near the basement membrane in pigment filled sacs. A detailed examination of numerous serial sections of the basal third of an ommatidium failed to produce any nuclei associated with the basal pigment "cells." It is probable that these are pigment filled sacs and until the cell body associated with these processes can be identified their origin will remain unknown.

Retinular cells

The cytoplasm of the retinula cells of C. hominovorax contains few organelles other than numerous mitochondria indicating a high level of energy utilization. Rough endoplasmic reticulum and golgi are poorly developed and generally consist of single isolated cisternae. Subrhabdomeric membranous channels and vesicles common in all the rhabdometric photoreceptors studied (see Discussion, Chapter I) are well developed in C. hominovorax.

The cytoplasm of the retinula cells appears so thin in many of the electron micrographs that the quality of the fixation was questioned. This condition is present in all the published micrographs of compound eyes (Wolken, 1971; Smith, 1968; Trujillo-Cenoz, 1965a, 1972; Boschek, 1971). The fixation and preparative procedures are not believed responsible for this condition since the pigment cells, asons, pseudocartridges, glial cells, and cartridges were well fixed. It is probable that after the eye is formed the retinula cells play a passive role. The presence of large numbers of mitochondria indicate a high level of energy utilization probably required for photochemical processes. After the eye is fully developed the primary function of the retinular cells is probably to maintain the rhabdomeres and provide the energy required for microvillar membrane bound photochemical reactions.

The relative orientation of the microvilli in the peripheral rhabdomeres of C. hominovorax is the same as that reported for Musca (Boschek, 1971), Chrysomia, Sarcophaga, and Lucilia (Trujillo-Cenoz, 1965a, 1972), and Calliphora (Smith, 1968). The microvillar orientation of the central cells (R7 and R8) is similar to that of Sympycnus lineatus (Dolichopodidae) (Trujillo-Cenoz & Bernard, 1972). In S. lineatus and C. hominovorax two populations of ommatidia are present based on the orientation of the central cell microvilli. Some of the ommatidia

have the typical orthogonal arrangement (Boschek, 1971; Melamed & Trujillo-Cenoz, 1968) while others have parallel microvilli (Trujillo-Cenoz & Bernard, 1972) (see Plates XVIII and XXIII A, B). The parallel arrangement of microvilli is believed to reduce the reception of polarized light in the plane perpendicular to the axis of the microvilli (Waterman *et al.*, 1969; Waterman and Horch, 1966; Eguchi & Waterman, 1966; Trujillo-Cenoz, 1972; Trujillo-Cenoz & Bernard, 1972).

Open rhabdom--Hemiptera and Diptera

In addition to the higher dipterans, several aquatic and semiaquatic Hemiptera have open rhabdoms. Notonecta (Ludtke, 1953) and the toad bug, Gelastocoris oculatus (Burton & Stockhammer, 1969) have open rhabdom eyes. Hemipteran eyes are of the eucone type. In eucone eyes the dioptric apparatus consists of a lens and 4 cone cells. In the pseudocone eyes characteristic of higher dipterans an extracellular pseudocone replaces the 4 cone cells. In spite of this basic difference several striking similarities are present when the eyes of Cochliomyia, and other higher dipterans, are compared with the eyes of Gelastocoris and Notonecta.

The cone cells of Hemiptera and the Semper cells of the dipterans have few organelles other than microtubules. In the dipterans the function of the cone cells is performed

by an extracellular pseudocone. It is very possible that the Semper cells of Cochliomyia and higher dipterans were derived from cone cells in a eucone ancestor. The fact that the lower Diptera have eucone eyes (Brammer, 1970) tends to support this view. If this is correct the pseudocone developed as an evolutionary advance that paralleled the reduction of the 4 cone cells.

The cone cells in Gelastocoris are surrounded by 2 primary pigment cells anatomically similar in structure to the primary pigment cells in the higher Diptera (Trujillo-Cenoz, 1972) that form the pseudocone cavity. I believe pseudocone eyes are a specialized type of eucone eye and the Semper cells are homologous with the cone cells.

The organization of the retinula cells in Gelastocoris (Hemiptera) and Cochliomyia (Diptera) is very similar. Both have 6 peripheral and 2 central rhabdomeres. The microvilli of the 2 central cells of Gelastocoris are orthogonal as are those of most higher dipterans (Trujillo-Cenoz, 1972).

An electron micrographic study of the cone cells in several species of eucone insects and the embryological development of the pseudocone and Semper cells in dipteran eyes will be needed to determine positively if the pseudocone eye is an advanced form of eucone eye.

Irradiated Cochliomyia hominivorax

Irradiated flies received from the rearing facility in Mission, Texas, were abnormal in a number of respects. The most common abnormality was a large increase in the number and size of vacuoles in the retinular cell cytoplasm. The cytoplasm was highly vacuolate and appeared poorly fixed but the fixation is not believed responsible for the abnormalities encountered. Eyes of Anastrepha suspensa (Tephritidae) were fixed concurrently with the irradiated screwworm fly eyes. The eyes of Anastrepha were well fixed and exhibited none of the abnormalities in the irradiated screwworm flies.

A large increase in the number of tracheoles near and between the retinular cells was also observed in the irradiated eyes (Plate XXII B, Plate XXI B, C).

Abnormally shaped rhabdomeres, rhabdomeric microvilli, and central cell transitions (Plates XXII, XXIII, and XXIV) were not consistently observed but were present in many of the individuals examined. The irradiated flies were highly variable on an individual and weekly basis. Eichenbaum and Goldsmith (1968) published an electron micrograph of an abnormal central cell transition zone in Musca eyes transplanted as imaginal discs into the abdomen of a larva. This abnormal transition zone contains 8 rhabdomeres and is similar in some respects to Plate XXIV C although no

bridge is present between R7 and R8. Eichenbaum and Goldsmith (1968) misnumbered the retinular cells (their No. 1 is actually No. 8) and stated that their electronmicrograph shows the "typical arrangement of photoreceptor cells" which is unfortunately incorrect.

The abnormalities observed in the flies that were irradiated as pupae are of an unknown origin. Unirradiated flies reared in Fargo, North Dakota, and irradiated flies from Mission, Texas, were reared on an artificial diet, "nutria" (Hoffman, personal communication; Holt, personal communication). The observed abnormalities in the flies reared in Mission, Texas, may be due to irradiation damage. The possibility of dietary insufficiencies inducing the abnormalities cannot be dismissed. Moore (1974) believes that a true wild type-fly has not been produced even on the best available meat and blood diets used. He feels the accumulation of waste products from the flies may cause many quality problems in a mass-rearing program.

Insects reared on artificial diets over many generations often deviate markedly from their wild counterpart. Agee (in press) has demonstrated considerable differences in the eye of Anastrepha suspensa due to the quality of their diet. Anastrepha reared on a superior diet were as much as 10 times more responsive to visual stimulation than those reared on other artificial diets.

CHAPTER III

THE SPECTRAL SENSITIVITY OF THE COMPOUND EYE OF Cochliomyia hominivorax

The importance of the screwworm fly has already been described in the previous chapter. The following research was done in part to substantiate the microanatomical findings and determine if there were any neurophysiological abnormalities associated with the fly.

Literature Review

The spectral sensitivity of the compound eyes of only a small number of insects has been determined using electrophysiological methods. Spectral sensitivities for insects representing only 7 orders have been determined. Spectra for the following have been published: Lepidoptera, Heliothis zea (Boddie), H. virescens (F.) (Agee, 1973) and Manduca sexta (L.) (Hoglund & Struve, 1970); Coleoptera, Dineutes ciliatus (Forsberg) (Bennet, 1967); Hymenoptera, Apis mellifera L. (Goldsmith, 1960, 1961); Odonata, Libellula luctuosa Burimerster (Ruck, 1965) and Libellula needhami Westfall (Horridge, 1969) and Hemiptera, Notonecta spp. (Bennett & Ruck, 1970).

The entire subject of insect vision has been covered in a book by Mazoklin-Porshnyakov (1969). Burkhardt (1964) and Goldsmith (1964) have written review articles treating electrophysiological studies on the insect compound eye and insect vision.

The most widely studied eye in terms of spectral sensitivity is that of Calliphora erythrocephala Meig. It has been the subject of many detailed electrophysiological and behavioral vision studies along with 2 mutant eye types, the "chalky" eye and the "white-apricot" eye. These 2 mutant forms have been used to determine the effect of screening pigments on the spectral sensitivity, particularly the presence of a red receptor in the flies.

Early studies of the spectral sensitivity of C. erythrocephala reported 3 peaks (Autrum & Stumpf, 1953; Walther & Dodt, 1959; Mazoklin-Porshnyakov, 1960). The most prominent peak was in the green, around 490 nm, a second peak occurred around 350 nm in the ultraviolet, and a third in the red region, about 620 nm. Subsequent studies on the 2 mutant strains of Calliphora lacking eye pigments, the "chalky" mutant and the "white-apricot" mutant, failed to produce a peak in the red near 620 nm. The first study by Autrum (1955) on the "white-apricot" mutant, lacking brown-red pigments, produced only 2 peaks, 350 nm and 490 nm. A second study (Hoffman & Langer, 1961) on the "chalky" mutant, lacking all eye pigments,

also produced 2 peaks, 1 at 350 nm and another at 500 nm.

Intracellular recordings of single receptor cells produced evidence indicating that the red peak was an artifact (Burkhardt, 1962, 1964). Burkhardt (1964) postulated that the 620 nm red pseudopeak is the result of differential absorption by the screening pigment. He postulates that the screening pigment does not absorb at 620 nm allowing extra light to pass and stimulate the receptor cells. This could account for a mass response in a spectral region where screening pigment does not absorb causing a false peak to appear.

The spectral sensitivity of single photoreceptor cells of wild-type Calliphora agree with the spectral sensitivity of the "chalky" mutant (Burkhardt, 1964). All the photoreceptor cells of the wild-type flies investigated had 2 peaks (Burkhardt, 1962, 1974). One peak is approximately 350 nm in the ultraviolet. The second peak can be in 3 different positions, 490 nm, 470 nm, or 521 nm. Approximately 80% of the receptor cells, the so-called green type, have a peak at 490 nm. Two other cell types, the yellow-green and the blue type, account for approximately 20% of the receptor cells. The yellow-green type has a peak at 470 nm and the blue type has a peak at approximately 521 nm. The mean peak of these 3 cell types

will fall at about 496 nm when large numbers of photoreceptor cells are plotted (Burkhardt, 1964).

An ommatidium illuminated by scattered light or reduced light exhibits an interesting spectral shift. The ultraviolet peak at 350 nm remains but the 490 nm peak shifts to approximately 515 nm and a 620 nm peak appears (Burkhardt, 1962, 1964; Autrum, 1955). As the intensity of light is increased the 620 nm peak disappears and the visible peak returns to 490 nm.

In Musca domestica L. 2 sensitivity peaks are present at 350 nm and 490 nm (Goldsmith & Hernandez, 1968). Studies on a white-eyed mutant of M. domestica failed to produce evidence of a red receptor (Goldsmith, 1965).

An alternate approach to the determination of spectral sensitivity is the use of optomotor responses. An optomotor response is a behavioral response initiated by an insect to a patterned stimulus. A rotating striped pattern was presented Phormia regina Meig. in spectral colors. The yawing force or torque developed by the fly in fixed flight was used as a measure of the optomotor reaction to various spectral colors and the spectral sensitivity was calculated. The sensitivity curve had 2 peaks, 1 in the ultraviolet at 353 nm and the other in the green at 490 nm (Kaiser, 1968; Kaiser & Liske, 1972).

Spectral sensitivity experiments either electro-physiological or optomotor have shown 3 peaks of sensitivity in flies closely related to C. hominovorax, an ultraviolet peak near 350 nm, a green peak at approximately 490 nm, and a red "pseudopeak" at 625 nm.

Methods and Materials

Screwworm flies, Cochliomyia hominovorax, were shipped to Gainesville, Florida, by air mail from H. C. Hoffmann, USDA, APHIS, Mission, Texas, as irradiated pupae (ca 7 Krad). These flies were mass reared for release in the screwworm fly eradication program and were received in the release container. Approximately 200 pupae were placed in a holding cage (15 x 30 x 60 cm) consisting of tube gauze stretched over an aluminum frame. A mixture of honey and water was provided the newly emerged flies as a source of sugar and water. The flies were held in a dark cabinet to minimize damage to the wings caused by the flies striking against the cage when flies were held on a normal light-dark regimen.

Flies 8 days of age were used for spectral sensitivity experiments and 3-day-old flies were used for the weekly experiments to measure the variation in visual sensitivity. Equal numbers of male and female flies were selected at random from the cage for testing. Approximately

60-70% of the flies set up for testing from some weekly shipments had very low visual sensitivities, some as low as 50 μ V. Only flies that required a neutral density attenuation of .6 or higher at a wavelength of 490 nm for a criterion response were selected for testing over the complete wavelength range, 350 nm to 650 nm.

The flies used for experimentation were immobilized on a wax block with 3 "U"-shaped wire clips. One clip was placed behind the head, another over the thorax and wings, and the third held the abdomen.

Electrical contact with the photoreceptor cells was made by positioning an electrosharpened stainless steel electrode (Agee, 1971) into each eye. The tip diameter of the recording electrode was approximately 1.7 microns and was placed into the right eye. The indifferent electrode was placed into the nonilluminated left eye. Both electrodes were placed in the dorsal hemisphere of the eye behind the vertical midline. The electrodes generally penetrated between the corneal facets and were placed approximately 50-100 microns below the surface of the eye. Following placement of the electrodes the eyes were allowed to dark adapt for 20 minutes prior to testing. Preliminary tests showed that visual sensitivity did not increase with additional time. Cochliomyia hominivorax eyes were found to fully dark adapt in about 15 minutes and the extra 5 minutes were added to insure complete dark adaptation.

The electrodes were connected push-pull to a Grass Model P-6 preamplifier with an amplification of 500X. The electrophysiological response was displayed on a Tektronix Model 565 dual trace oscilloscope. The upper beam was connected to a photocell that monitored the flash duration and the lower beam displayed the summed electrical response of the illuminated photoreceptor of the eye.

Optical Stimulation

The stimulating light source was a Bausch and Lomb No. 33-86-39-01 tungsten (quartz iodide) coil filament lamp. This lamp provided a continuous spectrum from the near ultraviolet to the infrared (300 nm to 700 nm). The lamp was operated at a voltage of 120 V AC and an amperage of 400 ma monitored with meters.

Monochromatic light was obtained with a Bausch and Lomb grating monochrometer Cat. No. 33-86-07 that provides a continuous spectrum from the ultraviolet to the infrared wavelengths. The purity of the light emitted from the exist slit is a product of the dispersion of the system and the width of the entrance and exit slits utilized. A slit width combination that gave a 10 nm bandpass was used. The entrance slit was 2.3 mm and the exit slit was set at 1.35 mm (Agee, 1973).

The light was chopped with a mechanical vane attached to a solenoid controlled by a Grass S-4 stimulator. Light

flashes of 235 milliseconds duration at a rate of 1 per 2.5 seconds were used.

Filters were utilized to control the quality and quantity of light delivered to the illuminated eye. Selected Corning filters were used to block second and third order wavelengths characteristically produced by grating systems. The higher orders are produced at integral multiples of the first order wavelengths. The blocking filters used were Corning No. 1-69 utilized in the wavelength range 350-700 nm, No. 5-59 at wavelengths of 365-480 nm and No. 4-65 used from 500-700 nm. Appendix A illustrates the percent transmittance of the various filters.

The equal response method for determining spectral sensitivity was used. The intensity of light stimuli delivered to the insect was regulated at each wavelength with a neutral density wedge. Light was attenuated to provide a criterion response defined for the purpose of this study as a 1 cm negative deflection ("on" response) on the oscilloscope screen. A response of 250 μ V was necessary to provide the criterion response. The neutral density wedge is a circular quartz iconel filter calibrated continuously from 0 neutral density (ND) to a ND of 2. Due to the low visual quality of the irradiated flies a ND of 1.5 was the highest value used compared with a 2.5 ND of the considerably smaller eye of Anastrepha suspensa.

(*Agee, unpublished). The wedge delivered a 90% attenuation of light at a ND of 1.0 and a 50% attenuation at a ND of 0.3. Light radiation was conducted from the monochrometer to a light-proof box through a tube painted internally with a flat black paint that absorbed the incident light (Agee, 1972). Flies were tested in an electrically shielded light-proof Faraday cage. The light-proof box contained a pair of micromanipulators and a dissecting microscope to aid in positioning the indifferent and recording electrodes.

The optical system was calibrated with a 12 junction bismuth-silver thermopile (Eppley) and a millimicrovolt meter. Most ERG studies have dealt with undefined relative units that are of limited value due to the problem encountered when attempting to relate two different studies or insects. Absolute units, watts per cm^2 , were calculated at full light intensity at each wavelength tested. Measurement of absolute units is attainable by calibration with a thermopile (comparable to National Bureau of Standards lamps).

The intensity of light measured with the thermopile and microvolt meter at 530 nm used in the weekly variance tests was calculated to be $2.63 \mu \text{ watts}/\text{cm}^2$ at full intensity. The value from the calibration for the intensity of light at a particular wavelength in watts/cm^2 was

multiplied by the percent attenuation of the ND wedge necessary to produce the criterion response. The reciprocal of this electrical response was graphically presented to show the spectral sensitivity of the screwworm fly eye.

Results

The average spectral sensitivity of 9 C. hominovorax in the spectral range of 350 nm to 650 nm is presented in Figure 5.

Three peaks of sensitivity are present. There is a peak in the ultraviolet region presumably at 350 nm. Only a portion of this peak is present since it falls on the first wavelength tested. The second and largest peak is present in the green region of the spectrum at 490 nm. A third smaller peak is present near 625 nm in the red region of the spectrum.

The visual sensitivity of 6 weekly shipments of irradiated screwworm flies as measured by the ERG at 530 nm is shown in Figure 6.

Discussion

The spectral sensitivity curve for irradiated C. hominovorax as determined by electrophysiological techniques is very similar to those previously reported for Calliphora (Walther & Dodt, 1959; Autrum & Burkhardt, 1961; Burkhardt, 1962, 1964; Mazoklin-Porshnyakov, 1960), Musca domestica

Figure 5. Spectral sensitivity curve of the dark-adapted compound eyes of Cochliomyia hominivorax. Each point is the mean for 9 insects.

5.

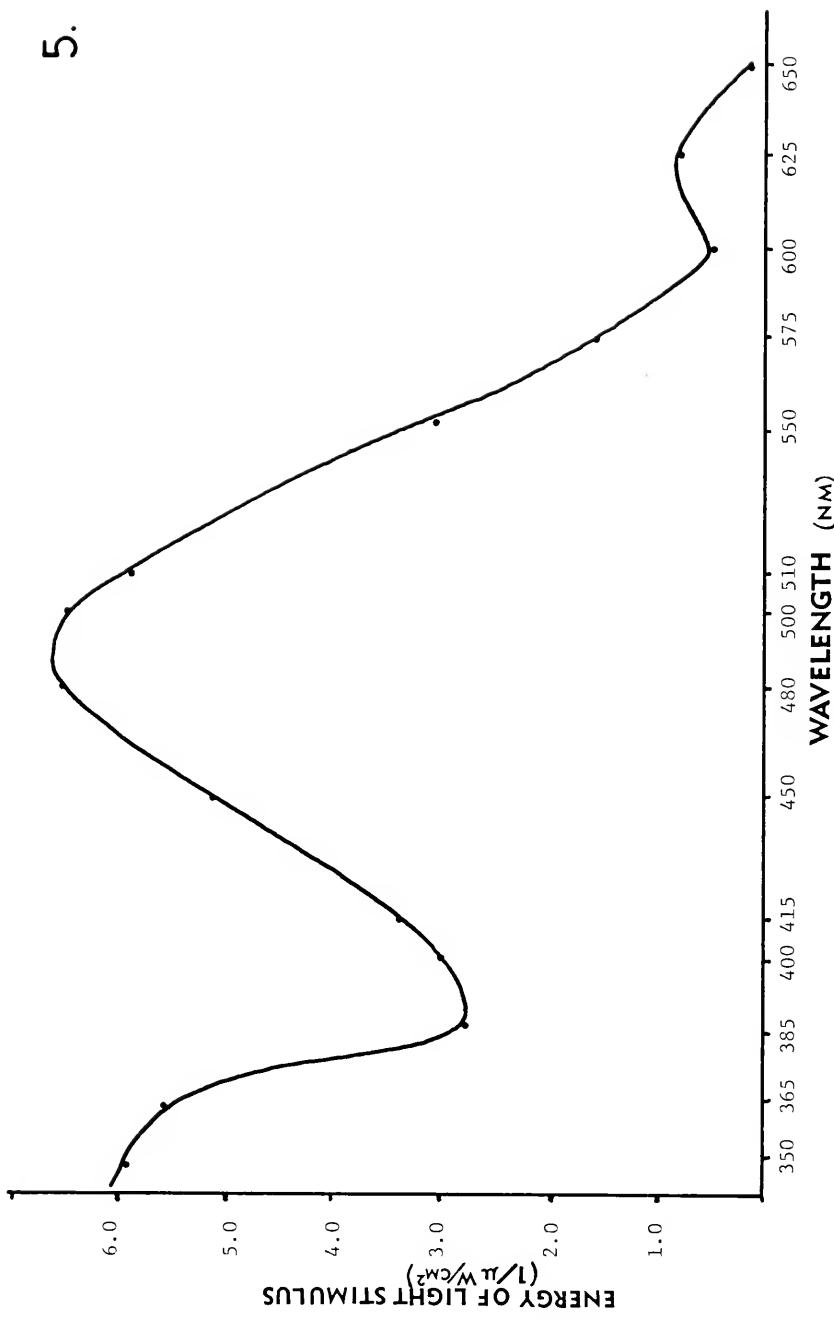
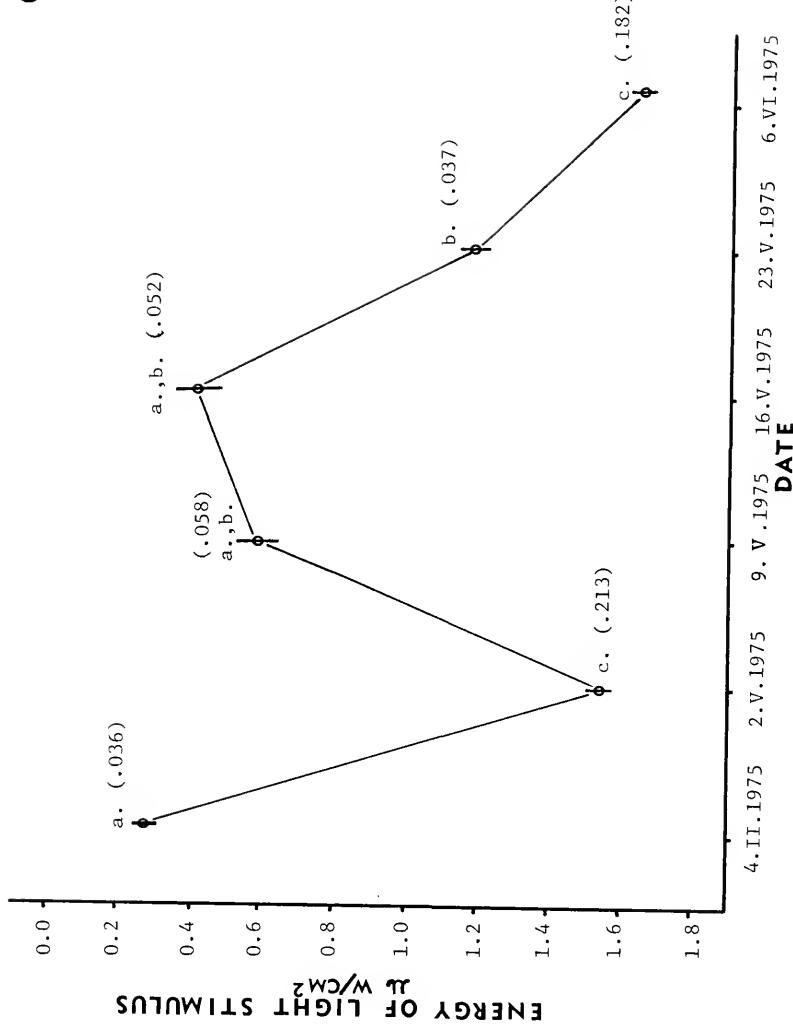


Figure 6. Weekly visual sensitivity of irradiated Cochliomyia
hominivorax. Each point represents the mean for 10
insects, 5 males and 5 females. Vertical lines indicate
the 95% confidence interval for the mean and the standard
error of the mean is shown in parentheses. Means indi-
cated by the same letter are homogeneous ($P = 0.01$,
Duncan's multiple range test).

6.



(Mazoklin-Porshnyakov, 1960; Goldsmith & Fernandez, 1968), and Phormia regina (Kaiser, 1968). Three peaks are present in all 4 species, a UV peak at approximately 350 nm, a larger peak in the green range near 490 nm, and a third peak at 625 nm. The third peak in the red region is probably a "pseudopeak" resulting from the lack of absorption by screening pigments around 625 nm. (Goldsmith, 1965; Burkhardt, 1962, 1964; Autrum, 1955; Hoffman & Langer, 1961).

An unpublished electrophysiological study of the spectral sensitivity of 4 strains of reared irradiated C. hominovorax showed 2 peaks, 1 at 550 nm (blue-green) and 1 at 630 nm (red) (Holt, unpublished report). These results are not consistent with prior work on related genera or the results of this study. No ultraviolet peak (350 nm) was reported since the spectra tested, 490 nm to 710 nm, did not include the UV portion of the spectra. The 630 nm red peak is probably a "pseudopeak" as discussed above. The primary sensitivity peak at 550 nm in all 4 strains is unexpected and unexplained. Scattered or reduced light has been reported to shift the green peak of sensitivity in flies from 490 nm to about 515 nm (Burkhardt, 1962). The criterion response used by Holt was 300 μ volt which was higher than the 250 μ volt criterion utilized in this study.

The visual sensitivity measured by the ERG at 530 nm exhibited considerable weekly variation. The highest weekly mean sensitivity was approximately 10 times more sensitive than the lowest weekly sensitivity. Visual quality has been demonstrated as a means of assessing the quality of mass-reared insects (Agee & Park, 1975). The wide variance of weekly values indicates considerable difference in the visual quality of flies released. Visual quality in Cochliomyia hominovorax may therefore serve as a means of determining the quality of the flies being reared and released. This area of screwworm fly biology deserves more attention to determine if visual quality can be used successfully as a quality control measure in the screwworm release program.

The estimated production of the new screwworm plant near Tuxtla Guterrez, Chiapas, Mexico, and the Mission, Texas, screwworm facility is estimated to be approximately one-half billion flies per week (Bushland, 1974a,b). The success of a rearing program of this magnitude will require a means of assessing the quality of the flies produced. Visual quality could be useful as a tool in the screwworm program and more research along these lines should be undertaken.

CHAPTER IV

CONCLUSIONS

The research performed in this study has led to the following conclusions on the three areas investigated:

- A. The microanatomy of the eyes of the "lonestar tick," Amblyomma americanum.
1. Amblyomma americanum L. has a functional eye. The photoreceptor neurons possess all the microanatomical features common to other rhabdometric photoreceptors.
2. The eye of A. americanum is primitive and not secondarily reduced.
3. The eye of A. americanum is the first arhabdomate eye in the Arthropoda.
4. The photoreceptor neurons show striking affinities with the turbellarian Platyhelminthes (flatworms) and pulmonate Mollusca (snails). All three have arhabdomate eyes, terminal microvilli lacking microvillar interaction, and very similar organelles and cellular organization.

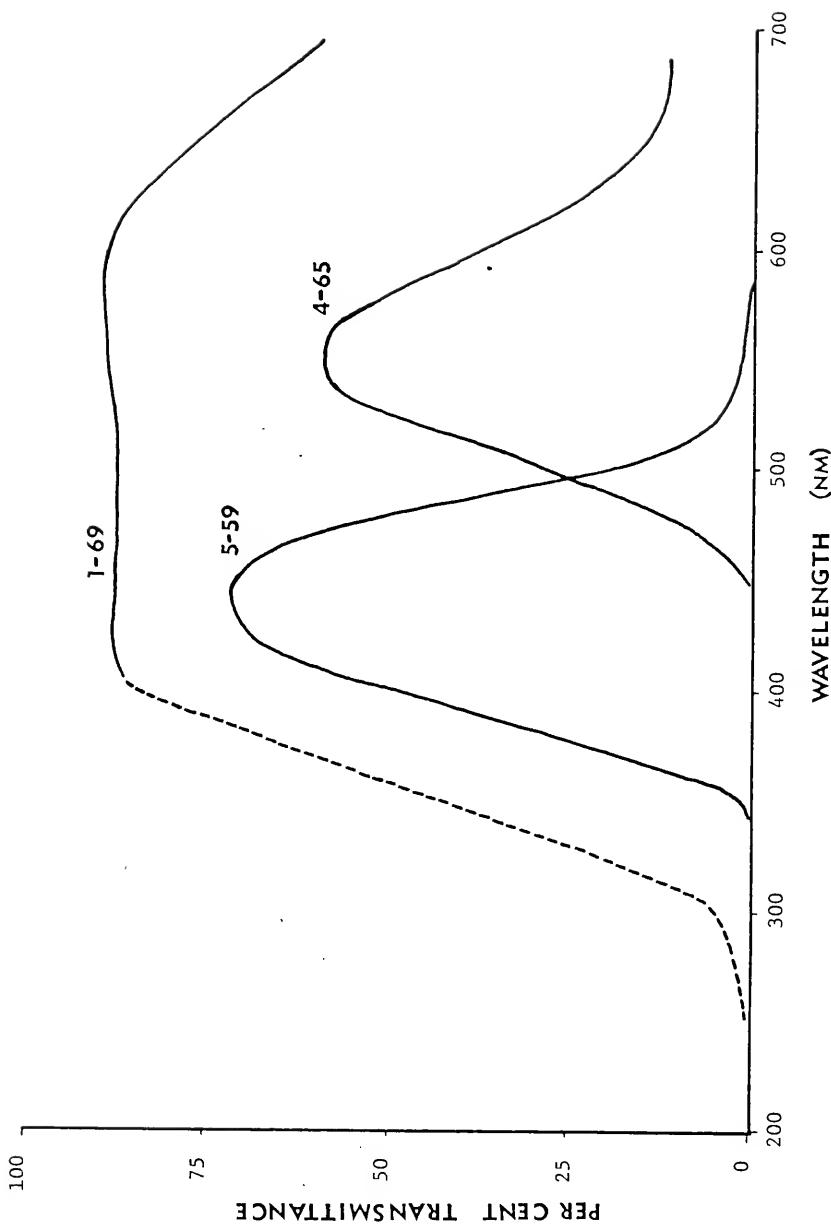
5. The affinities of the eyes of flatworms, snails, and ticks place the arthropods in the main line of rhabdomeric photoreceptor evolution. Evolutionary dendograms previously had placed the flatworms and snails outside the mainstream of evolution.
- B. The microanatomy of the eye of the "screwworm fly," Cochliomyia hominivorax (Coquerel).
 1. The microanatomy of the peripheral retina and lamina of unirradiated screwworm flies is very similar to the other genera of cyclorrhaphan Diptera studied.
 2. The Semper cells maintain the distal trapezoidal pattern of the open rhabdom as do the rhabdomere caps, desmosomal and gap junctions of the Semper cells and the retinular cells. The significance of this configuration is structural continuity and permits according to many scientists visual data processing.
 3. A 7-armed extension of the ommatidial cavity extends into the Semper cells. The ommatidial cavity is fluid filled and contains an unknown electron dense material.
 4. "Pigment granules" of previous authors are actually vacuoles filled with crystals of pigment.

5. Retinular cells have no apparent metabolic functions and appear to be basically light transducers.
 6. Morphological evidence indicates two types of polarized light reception.
 7. The cone cells of eucone eyes and the Semper cells of pseudocone eyes appear to be homologous. The pseudocone eye is believed to be an advanced type of eucone eye.
 8. Irradiated flies showed a number of abnormalities.
 - a. Obconical rhabdomeres
 - b. highly vacuolate cytoplasm
 - c. increased number of trachea at all levels in the peripheral retina
 - d. abnormally shaped microvilli
 - e. decrease in number of microvilli
 - f. abnormal central cell transition zone.
 9. Although both irradiated and unirradiated flies were reared on artificial "nutria" diet, nutritionally induced abnormalities cannot be discounted.
- C. Spectral Sensitivity Studies.
1. Irradiated flies all demonstrated poor visual quality. Often 70% or more of a test group failed to give an adequate criterion response.

2. Considerable weekly variance in visual sensitivity was determined.
3. The spectral sensitivity curve of C. hominovorax was 3 peaks. The largest peak is at 490 nm. A second peak of sensitivity is present in the ultraviolet region at 350 nm and a third "pseudopeak" is present at 625 nm. The large 490 nm peak would indicate a strong response to the color green. Confirmation of this research should be done with the optomotor technique.

APPENDIX

Table 1. Per cent transmission of filters used to block second and third order wavelengths in the spectral sensitivity and visual sensitivity experiments.



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BIOGRAPHICAL SKETCH

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William Avery Phillis III is the father of two children, Emily Love Phillis and Colby Anne Phillis.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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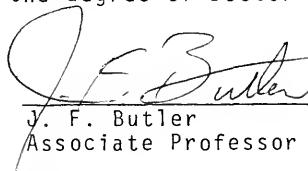
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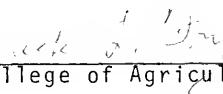


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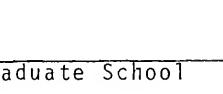
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